

AUTO/ALLO-IMMUNE DEFENSE RECEPTORS FOR THE SELECTIVE TARGETING
OF ACTIVATED PATHOGENIC T CELLS AND NK CELLS

[0001] This application claims priority to U.S. Provisional Patent Application 62/662,817, filed April 26, 2018, which is incorporated by reference herein in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED
RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under P50 CA126752 awarded by National Institutes of Health and the National Cancer Institute. The government has certain rights in the invention.

TECHNICAL FIELD

[0003] Embodiments of the disclosure include at least the fields of immunology, cell biology, molecular biology, and medicine.

BACKGROUND

[0004] Unwanted activation of T- and NK- cells often promotes life-threatening allo-immune reactions in patients receiving transplants or third party-derived therapeutic cells, leading to rejection of a transplanted organ/tissue or development of graft-versus-host disease (GvHD). Likewise, unwanted activation of autoreactive T-cells can lead to devastating autoimmune conditions, such as diabetes mellitus, autoimmune colitis, and multiple sclerosis. Currently, most of these diseases are not curable because of the inability to selectively eliminate pathogenic T cells. Instead, the patients are often treated with immunosuppressive drugs that render them immunodeficient and therefore susceptible to infections and malignant transformations.

[0005] The present disclosure provides solutions for a long felt need in the art of safe and effective tissue transplantation and adoptive cell transfer, including utilizing off-the-shelf cells, by enhancing their ability of the transferred cells to control pathogenic conditions because of unwanted activation of the immune system.

BRIEF SUMMARY

[0006] The present disclosure is directed to compositions and methods related to cells utilized for adoptive transfer to control pathogenic conditions due to immune activation. The composition and methods apply to autologous and allogeneic cells. Although some steps may be taken to reduce the reactivity of allogeneic cells in the recipient individual, such cells would still be targeted by the immune system of the recipient (primarily T- and NK-cells), which would recognize them as foreign leading to rejection and limiting therapeutic benefit.

[0007] The present disclosure overcomes this problem by modifying adoptive therapy cells to target activated pathogenic T, NK-T, and NK cells to prevent or treat medical conditions associated with their presence. In particular embodiments, the compositions and methods utilize adoptive T-cell transfer with cells that express receptors that selectively target pathogenic T cells while sparing resting T cells. In specific embodiments, the adoptive T-cells for transfer are engineered to express chimeric molecules that target pathogenic T cells that express certain target molecules whose presence on T cells is indicative of pathogenic T cells. In particular embodiments the disclosure concerns auto/allo-immune defense receptors (ADRs) for the selective targeting of pathogenic T-cells.

[0008] Particular embodiments of the disclosure include methods of protecting engineered allogeneic T cells from elimination in a host individual by providing to the individual cells armed with ADRs. Embodiments also include methods that avoid allo-immune reactions in individuals receiving tissue or organ transplants, for example.

[0009] In particular embodiments, cells encompassed by the disclosure have been modified or can be modified to allow them to survive in a recipient, including an allogeneic recipient. In specific cases, cells for adoptive cell therapy (including T cells, NKT cells, and so forth) are suitable for being utilized “Off-the-shelf”, which herein refers to cells kept in a repository, or bank, that may be provided (with or without further modification) to an individual in need thereof for a specific purpose. The individual in many cases is not the individual from which the cells were originally derived. The cells utilized in such a manner may be pre-manufactured to express an ADR, although in some cases the cells are obtained from a bank and afterwards are modified to express an ADR. The banked cells may or may not also express a CAR or recombinant TCR, or the cells obtained from the bank may afterwards be modified to express a CAR or recombinant TCR. Such practices allow for ease of use of third party-derived

therapeutic cells without immune rejection by a host and without having to manufacture a patient-specific produce every time one is needed.

[0010] In particular embodiments, there is an isolated polynucleotide, comprising sequence encoding: (1) one or more of an OX40-specific ligand, a 4-1BB-specific ligand, CD40L-specific ligand, or functional derivatives thereof; that is operably linked to (2) a signaling domain promoting T-cell activation. The polynucleotide may comprise an OX40-specific ligand, a 4-1BB-specific ligand, or a CD40L-specific ligand. The OX40-specific ligand may be OX40L, an antibody that targets OX40, an OX40L-Fc fusion, or a combination thereof, or any other engineered protein capable of specific binding to OX40. The 4-1BB-specific ligand may be 4-1BBL, an antibody that targets 4-1BB, a 4-1BBL-Fc fusion, or a combination thereof, or any other engineered protein capable of specific binding to 4-1BB. The CD40L-specific ligand may be CD40, an antibody that targets CD40L, a CD40-Fc fusion, or any other engineered protein capable of specific binding to CD40L or combination thereof. In at least certain cases, the polynucleotide further comprises sequence that encodes a spacer between (1) and (2), such as between 10 and 220 amino acids in length, for example. The spacer may have sequence that facilitates surface detection with an antibody, such as the spacer being detectable with an anti-Fc Ab. The spacer may comprise an IgG Fc portion.

[0011] In particular embodiments, polynucleotides of the disclosure may further encode a chimeric antigen receptor, a T-cell receptor, or both. The polynucleotide may be in any form including present on a vector, such as a viral vector (retroviral vector, lentiviral vector, adenoviral vector, or adeno-associated viral vector) or non-viral vector (plasmid, transposon, *etc.*). In particular cases, the polynucleotide is present in a cell, including a eukaryotic cell or a bacterial cell. The cell may be an immune cell, such as a T cell. The cell may be engineered. The cell may comprise one or more chimeric antigen receptors (CARs) and/or one or more engineered T cell receptors (TCRs).

[0012] Polypeptides expressed by any polynucleotide encompassed by the disclosure are included as part of the disclosure. In particular embodiments there is a polypeptide, comprising: (1) one or more of an OX40-specific ligand, a 4-1BB-specific ligand, and CD40; that is operably linked to (2) a signaling domain promoting T-cell activation. The signaling domain promoting T-cell activation may be from CD3 zeta subunit, DAP12, an Fc receptor, or a combination thereof.

[0013] Any cell encompassed by the disclosure is part of the disclosure. In specific embodiments, any chimeric receptor-expressing cell is part of the disclosure, including cells, comprising any polynucleotide contemplated herein and/or any polypeptides contemplated herein. The cell may be an engineered cell. The cell may be an immune cell, such as a T cell, including a CAR-transduced T cell and/or a T cell receptor (TCR)-transduced T cell. In specific embodiments, the cell is engineered to lack endogenous expression of one or more genes, such as lack one or more of 4-1BB, OX40 and/or CD40L. The cell may be engineered using CRISPR/Cas9, zinc finger nucleases, TALE nucleases, or meganucleases. Alternatively, the cell may be engineered to prevent surface expression of ADR ligands, for example, by trapping the ADR ligand with a specific antibody or a receptor anchored in the endoplasmic reticulum or another intracellular compartment.

[0014] In one embodiment there is a method of avoiding rejection of allogeneic cells, tissue, or organs in an individual, comprising the step of delivering to the individual an effective amount of allogeneic immune cells expressing an engineered chimeric receptor that comprises an extracellular domain that targets compounds that are selectively present on activated T cells and that comprises CD3 zeta, wherein the delivering step results in the following in the individual: (1) inhibition of endogenous alloreactive T cells in the individual; and/or (2) suppression of NK cell activation in the individual. In specific embodiments, the allogeneic cells are the allogeneic immune cells expressing the chimeric receptor. The allogeneic cells may express a chimeric antigen receptor and/or an engineered T cell receptor. The allogeneic immune cells may be delivered to the individual before, during, and/or after tissue and/or organ transplantation in the individual. In specific cases, the activated T cells are pathogenic T cells.

[0015] In one embodiment, there is a method of selectively targeting activated T cells in an individual, comprising the step of providing to the individual an effective amount of cells expressing an engineered chimeric receptor, said chimeric receptor comprising: (1) an extracellular domain that targets compounds that are selectively present on activated T cells; and (2) a signaling domain promoting T-cell activation. The signaling domain promoting T-cell activation may be derived from CD3 zeta subunit, DAP12, an Fc receptor, any ITAM-comprising sequence, or a combination thereof. In specific cases, the activated T cells are pathogenic T cells.

[0016] In a certain embodiment, there is a method of preventing or treating a medical condition related to activated T cells in an individual, comprising the step of delivering to the individual an effective amount of immune cells expressing an engineered chimeric receptor that selectively targets said activated T cells, said chimeric receptor comprising: (1) an extracellular domain that targets compounds that are selectively present on activated T cells; and (2) a signaling domain promoting T-cell activation. The medical condition may be an autoimmune disorder, such as graft rejection, graft-versus-host disease, type I diabetes, multiple sclerosis, autoimmune colitis, or a combination thereof, for example.

[0017] In one embodiment, there is a method of avoiding NK cell-mediated host rejection of allogeneic T cells, tissues, or organs in an individual, comprising the step of providing to the individual an effective amount of immune cells expressing an engineered chimeric receptor that comprises an extracellular domain that targets compounds that are selectively present on activated T cells and that also comprises a signaling domain promoting T-cell activation. The immune cells expressing the engineered chimeric receptor are the allogeneic T cells, in certain cases. The immune cells may express a chimeric antigen receptor and/or an engineered T cell receptor. The amount of immune cells expressing the engineered chimeric receptor that are provided to the individual may be in a range of 10^2 - 10^{12} per m^2 . The cells expressing the chimeric receptor may be provided to the individual systemically or locally. The immune cells may be T cells. The immune cells may be delivered to the individual once or more than once.

[0018] The foregoing has outlined rather broadly the features and technical advantages of the present disclosure in order that the detailed description that follows may be better understood. Additional features and advantages will be described hereinafter which form the subject of the claims herein. It should be appreciated by those skilled in the art that the conception and specific embodiments disclosed may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes of the present designs. It should also be realized by those skilled in the art that such equivalent constructions do not depart from the spirit and scope as set forth in the appended claims. The novel features which are believed to be characteristic of the designs disclosed herein, both as to the organization and method of operation, together with further objects and advantages will be better understood from the following description when considered in connection with the accompanying figures. It is to be

expressly understood, however, that each of the figures is provided for the purpose of illustration and description only and is not intended as a definition of the limits of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] For a more complete understanding of the present disclosure, reference is now made to the following descriptions taken in conjunction with the accompanying drawing, in which:

[0020] FIGS. 1A-1E. ADRs can be expressed on cell surface of immune cells and promote cytotoxicity against respective targets. (FIG. 1A) Schematic of ADR. GFP is optional (FIG. 1B) Expression of ADR on the cell surface (FIG. 1C) Expansion of ADR T cells after transduction (FIG. 1D) Cytotoxicity of ADR T cells against target cells expressing ADR ligands (FIG. 1E) Expansion of wild-type vs 4-1BB KO T cells expressing 4-1BB ADR and their cytotoxicity against 4-1BB+ targets showing that knocking out ADR ligand on T cells can further enhance expansion and cytotoxicity and demonstrating co-expression of ADR and its ligand on T cells is not required for ADR-T cell expansion or function

[0021] FIGS. 2A-2E. Selective expression of ADR ligands on activated T cells enables their selective elimination by ADR T cells. (FIG. 2A- FIG. 2C) Expression of ADR ligands on resting vs activated T cells after TCR stimulation. (FIG. 2D) Absence of cytotoxicity of ADR T cells against resting CD4+ and CD8+ T cells (FIG. 2E) Elimination of activated CD4+ and CD8+ T cells by ADR T cells after a 48h coculture.

[0022] FIGS. 3A-3F. Expression of 4-1BB ADR protects T cells from immune rejection in an MLR model (FIG. 3A) Representative dot plots showing TCRKO T cells co-expressing ADR are protected from rejection after coculture with allogeneic PBMC at a 1:10 ADR T:PBMC ratio. (FIG. 3B- FIG. 3C) Absolute counts of donor T cells and allogeneic T cells in the PBMC during coculture (FIG. 3D- FIG. 3F) same for virus-specific ADR T cells.

[0023] FIGS. 4A-4C. Expression of ADR protects allogeneic virus-specific T cells from immune rejection in a mixed lymphocyte reaction in vitro (FIG. 4A) Representative dot plots showing ADR VST are protected from immune rejection by recipient allogeneic PBMC (FIG. 4B- FIG. 4C) Absolute counts of recipient T cells and donor VST at various time points during MLR.

[0024] FIG. 5. ADR VSTs retain anti-viral function. ADR VSTs were cocultured with viral pepmix-pulsed monocytes, and monocyte counts indicated that they eliminated viral infected cells equally well compared to unmodified VSTs.

[0025] FIGS. 6A-6H. Activated NK cells upregulate ADR ligands and can be selectively targeted by ADR T cells (FIG. 6A- FIG. 6B) Expression of 4-1BB on resting vs activated NK cells (FIG. 6C) Residual counts of resting vs activated NK cells after 24hr coculture with of 4-1BB ADR T cells (FIG. 6D) ADR T cells lacking MHC are protected from immune rejection by allogeneic PBMC by controlling the expansion of NK cells (FIG. 6E) Absolute counts of donor T cells and allogeneic NK cells during coculture. (FIG. 6F) ADR T cells lacking MHC resist immune rejection by NK cells upon 48h coculture at a 1:1 E:T ratio. (FIG. 6G- FIG. 6H) ADR T cells control the expansion of alloreactive NK cells during MLR with PBMC, with absolute counts of NK cells plotted in H.

[0026] FIGS. 7A-7E. ADR expression protects allogeneic T cells from immune rejection in vivo (FIG. 7A) Schematic of the mouse model of immune rejection where mice were given T cells from an HLA-A2+ donor after a sublethal irradiation, followed by administration of allogeneic HLA-A2- T cells 4 days later. (FIG. 7B- FIG. 7C) Control T cells from the HLA-A2- donor were rejected by Day 18 while ADR-expressing cells were protected (FIG. 7C) Absolute counts of T cells from HLA-A2+ and HLA-A2- donors at various time points. (FIG. 7D) Modified in vivo model where instead of allogeneic T cells mice received whole PBMC (containing both T- and NK-cells) from donor 1. (FIG. 7E) Representative flow plots showing ADR T cells were protected from immune rejection and also protected mice from rapid onset of fatal GvHD.

[0027] FIGS. 8A-8E. Coexpression of CAR and ADR preserves functions of both receptors (FIG. 8A) Schematic representation of an immune cell co-expressing ADR and a CAR (FIG. 8B) Coexpression of CAR and ADR on the cell surface (FIG. 8C) Cytotoxicity of CAR-ADR T cells against NALM-6 (CD19+ CAR target) (FIG. 8D) Cytotoxicity of CAR-ADR T cells against activated T cells (ADR target) (FIG. 8E) Cytotoxic activity of CAR-ADR T cells against both targets upon simultaneous co-culture with both cell targets.

[0028] FIGS. 9A-9E. CAR-ADR T cells are protected from immune rejection and exert potent anti-tumor activity (FIG. 9A) Schematic of the mouse model. Mice received allogeneic T cells from Donor 1 and b2mKO NALM6 24hr apart, followed by a single dose of CAR-ADR T

cells from Donor 2. (FIG. 9B) Kinetics of T cells from Donor 2 in peripheral blood (FIG. 9C) Kinetics of Donor 1 T cells in the experimental groups (FIG. 9D) Leukemia burden in mice (FIG. 9E) overall survival of mice.

[0029] FIGS. 10A-10C. CAR-ADR T cells are protected from immune rejection and exert potent anti-tumor activity in a solid tumor model. (FIG. 10A) Schematic of the mouse model. Mice received allogeneic T cells from Donor 1 and b2mKO neuroblastoma cell line CHLA255 24hr apart, followed by a single dose of CAR-ADR T cells from Donor 2. (FIG. 10B) Donor 2 GD2 CAR T cells were rejected by D18, whereas CAR-ADR T cells resisted allogeneic rejection and persisted in peripheral blood. (FIG. 10C) Tumor burden in mice, * indicates xenogeneic-GvHD associated deaths in ATC+GD2 CAR T group.

[0030] FIGS. 11A-11E. TCR-knockout CAR-ADR T cells are protected from immune rejection and exert potent anti-tumor activity (FIG. 11A) Schematic of the mouse model. Mice received allogeneic T cells from Donor 1 and b2mKO NALM6 24hr apart, followed by a single dose of TCR-edited CAR-ADR T cells from Donor 2. (FIG. 11B) Kinetics of T cells from Donor 2 in peripheral blood (FIG. 11C) Kinetics of Donor 1 T cells in the experimental groups (FIG. 11D) Leukemia burden in mice (FIG. 11E) overall survival of mice.

[0031] FIGS. 12A-12D. ADR T cells protect mice against fatal xenogeneic GvHD (FIG. 12A) Schematic of the model (FIG. 12B) Expansion of FFLuc-labeled ADR T cells in vivo (FIG. 12C) Kinetics of weight gain/loss in mice (FIG. 12D) Overall survival of mice.

[0032] FIGS. 13A-13G. 2nd generation ADR with CD28 intracellular signaling domain (ADR.28zeta). (FIG. 13A) Structure of ADR.28zeta. (FIG. 13B- FIG. 13C) in vitro cytotoxicity of ADR.28zeta against target-expressing cell lines. (FIG. 13D- FIG. 13G) ADR.28zeta protected mice from xeno-GvHD. (FIG. 13D) Schematic of the model (FIG. 13E) Expansion of FFLuc-labeled ADR.28zeta T cells in vivo (FIG. 13F) Kinetics of weight gain/loss in mice (FIG. 13G) Overall survival of mice.

[0033] FIG. 14. Cytotoxicity of ADR-expressing cells in cancer. (left) Cytotoxicity of 4-1BB ADR-expressing T cells against HDLM-2 Hodgkin's lymphoma cells (right) Cytotoxicity of 4-1BB ADR-expressing T cells against K562 chronic myeloid leukemia (CML) cells. Absolute counts of tumor cells upon a 48h coculture at a 1:1 effector-to-target ratio is shown.

DETAILED DESCRIPTION

[0034] As used herein, the words “a” and “an” when used in the present specification in concert with the word comprising, including the claims, denote “one or more.” Some embodiments of the invention may consist of or consist essentially of one or more elements, method steps, and/or methods of the invention. It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein.

[0035] Throughout this specification, unless the context requires otherwise, the words “comprise”, “comprises” and “comprising” will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements. By “consisting of” is meant including, and limited to, whatever follows the phrase “consisting of.” Thus, the phrase “consisting of” indicates that the listed elements are required or mandatory, and that no other elements may be present. By “consisting essentially of” is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase “consisting essentially of” indicates that the listed elements are required or mandatory, but that no other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

[0036] Reference throughout this specification to “one embodiment,” “an embodiment,” “a particular embodiment,” “a related embodiment,” “a certain embodiment,” “an additional embodiment,” or “a further embodiment” or combinations thereof means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, the appearances of the foregoing phrases in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments.

[0037] The term “subject,” as used herein, generally refers to an individual in need of a therapy for a medical condition of any kind. A subject can be an animal of any kind. The subject can be any organism or animal subject that is an object of a method or material, including mammals, *e.g.*, humans, laboratory animals (*e.g.*, primates, rats, mice, rabbits), livestock (*e.g.*,

cows, sheep, goats, pigs, turkeys, and chickens), household pets (*e.g.*, dogs, cats, and rodents), horses, and transgenic non-human animals. The subject can be a patient, *e.g.*, have or be suspected of having a disease (that may be referred to as a medical condition), such as one or more infectious diseases, one or more genetic disorders, one or more cancers, or any combination thereof. The disease may be pathogenic. The subject may be undergoing or having undergone antibiotic treatment. The subject may be asymptomatic. The subject may be healthy individuals. The term "individual" may be used interchangeably, in at least some cases. The "subject" or "individual", as used herein, may or may not be housed in a medical facility and may be treated as an outpatient of a medical facility. The individual may be receiving one or more medical compositions *via* the internet. An individual may comprise any age of a human or non-human animal and therefore includes both adult and juveniles (*i.e.*, children) and infants and includes *in utero* individuals. The individual may be of any race and gender. It is not intended that the term connote a need for medical treatment, therefore, an individual may voluntarily or involuntarily be part of experimentation whether clinical or in support of basic science studies.

[0038] The term "engineered" as used herein refers to a molecule that is not present in nature and has been generated by the hand of man, such as by genetic recombination techniques standard in the art.

[0039] In the context of the present disclosure, an "effective amount" or a "therapeutically effective amount" refers to the amount of cells that, when administered to an individual, allows for targeting of activated T cells and/or alleviates the signs and or symptoms of a medical condition or prevents a medical condition. The actual amount to be administered can be determined based on studies done either *in vitro* or *in vivo* where the functional immune cells exhibit pharmacological activity against a medical condition.

I. Auto/Allo-Immune Defense Receptors and Compositions and Uses Thereof

[0040] The present disclosure encompasses synthetic chimeric receptor molecules that provide for selective targeting of activated T cells, including pathogenic T cells. The engineered molecules are synthetic and may be produced by recombinant technology. The molecules may be referred to as auto/allo-immune defense receptors that target activated T cells, including activated pathogenic T cells, including with high specificity.

[0041] In particular embodiments, auto/allo-immune defense receptors (ADRs) comprise an entity that targets one or more compounds that are upregulated on activated T cells. Although

the compound that is upregulated on activated T cells may be any one or combination thereof, in specific embodiments OX40, 4-1BB, and CD40L are upregulated on activated T cells and are the subjects of which the ADRs are targeting. The ADRs are present on allogeneic immune cells that are allogeneic with respect to the individual receiving the cells, in particular embodiments. In other instances, the ADRs are expressed on autologous T cells, xenogeneic cells, and/or synthetic cells.

A. Auto/Allo-Immune Defense Receptor (ADR) Molecules

[0042] ADR molecules are synthetic, non-natural, and produced by the hand of man and comprise at least (1) an extracellular domain that targets compounds that are selectively present on activated T cells (and in specific embodiments, the extracellular domain is a protein or functional fragment or derivative thereof that targets one or more compounds that are upregulated on activated T cells); that is operably linked to (2) a signaling domain promoting T-cell activation, including those derived from CD3 zeta subunit, DAP12, and Fc receptors, or another ITAM-comprising sequence, for example. The ADR molecule may comprise or consist of or consist essentially of elements (1) and (2). In at least certain cases, the ADR comprises one or more components of a Type I transmembrane protein and/or one or more components of a Type II transmembrane protein.

[0043] In specific embodiments, in the ADR molecule the extracellular domain comprises a protein that selectively binds an associated protein on an activated T cell. For example, the ADR extracellular domain may comprise a ligand for a receptor on an activated T cell, or the ADR extracellular domain may comprise a receptor for a ligand on an activated T cell.

[0044] In specific embodiments, in the ADR molecule the extracellular domain comprises a ligand for OX40, a ligand for 4-1BB, and/or CD40. These particular examples have associated proteins on activated T cells that are OX40, 4-1BB, and CD40L, respectively. In alternative embodiments, other particular compositions on the activated T cells are targeted. For example, one may target other activation markers that are upregulated on the cell surface of T cells (like CD69, CD25, CD71, *etc.*) can be targeted using a similar approach. In such cases, the corresponding ADR molecule would have a respective CD69, CD25, or CD71 ligand, or an antibody-derived targeting moiety, instead of 4-1BB/OX40-specific ligands.

[0045] In some cases, activated T cells are targeted that have upregulation of expression of OX40, in contrast to T cells that are not activated. To target these activated T cells, a ligand of OX40 could be utilized in the ADR to be able to target the activated T cells. In cases wherein a ligand for OX40 is utilized in the ADR, the ligand for OX40 may be any suitable ligand for OX40 including at least OX40L, an antibody (or functional fragment thereof) that binds to OX40, a fusion of Fc with OX40L, or functional derivatives or fragments thereof. OX40L may also be referred to as tumor necrosis factor (ligand) superfamily, member 4 (tax-transcriptionally activated glycoprotein 1, 34kDa), OX40L, CD252, TNFSF4, TXGP1, OX-40L, or gp34.

[0046] In some cases, activated T cells are targeted that have upregulation of expression of 4-1BB, in contrast to T cells that are not activated. To target these activated T cells, a ligand of 4-1BB could be utilized in the ADR to be able to target the activated T cells. In cases wherein a ligand for 4-1BB is utilized in the ADR, the ligand for 4-1BB may be any suitable ligand for 4-1BB including 4-1BBL, an antibody (or functional fragment thereof) that targets 4-1BB, a fusion of Fc with 4-1BBL, or functional derivatives or fragments thereof.

[0047] In certain cases, activated T cells are targeted that have upregulation of expression of CD40L, in contrast to T cells that are not activated. To target these activated T cells, a receptor for CD40L could be utilized in the ADR to be able to target the activated T cells. In cases wherein a receptor for CD40L is utilized in the ADR, the ADR may comprise CD40 (that may also be referred to as Bp50, CDW40, TNFRSF5, or p50), an antibody (or functional fragment thereof) that targets CD40L, or functional derivatives or fragments thereof.

[0048] In some cases, the ADR molecule comprises two or more extracellular domains to facilitate targeting of the activated T cells. Such combinations may enhance targeting of activated T cells generally or may allow for specific targeting of certain subsets of activated T cells. For example, the ADR may comprise both OX40L and 4-1BBL as extracellular domains in the same ADR molecule to allow for targeting of activated T cells that express either OX40 or 4-1BB. Such an example of a combination would selectively target activated T cells that express either OX40 or 4-1BB regardless of whether or not those activated T cells also express CD40L. Analogously, ADRs may comprise both CD40 and OX40L to target activated T cells that express either CD40L or OX40 regardless of whether or not those activated T cells also express 4-1BB.

[0049] In the ADR molecule, the extracellular domain may be operably linked to one or more components, including components that are part of the ADR molecule. One such component may be a protein that mediates downstream signaling during T cell activation. In particular embodiments the ADR comprises CD3zeta (also referred to as CD247, CD3-ZETA, CD3H, CD3Q, CD3Z, IMD25, T3Z, or TCRZ) or a functional fragment or derivative thereof. CD3zeta mediates downstream ITAM-derived signaling during T cell activation. Other ITAM-containing signaling domains may include those derived from DAP12, Fc receptors, other CD3 subunits, *etc.* The signaling domains may be non-covalently linked to the ADR via another domain.

[0050] In particular embodiments, the ADR comprises a spacer between the CD3 zeta and the extracellular protein that targets one or more compounds that are upregulated on activated T cells. In other cases, a spacer is not utilized. The spacer may comprise sequence that is inert or contributes substantially little or nothing with respect to any function the ADR may have, whereas in other cases the spacer comprises sequence that enhances a function of the ADR and/or allows it to be detectable and/or able to be targeted for inhibition, as examples. In specific embodiments, the spacer comprises encoded protein sequence that facilitates detection of cells that express the ADR. For example, the spacer may encode Fc region or fragments thereof that would allow for surface detection of the cells, such as using anti-Fc Abs. In particular embodiments, the spacer provides separation between the ligand binding domain and the membrane to avoid potential steric hindrances, such as those caused by the splicing of Type II transmembrane proteins (4-1BBL, OX40L) with the Type I ADR backbone (TM, signaling domains). The spacer may be of any suitable length, including about 10-220 amino acids as an example. The spacer length may be in a range of 10-220, 10-200, 10-150, 10-100, 10-50, 25-200, 25-150, 25-100, 25-75, 25-50, 50-200, 50-150, 50-125, 50-100, 50-75, 75-200, 75-150, 75-100, 100-200, 100-175, 100-150, 100-125, 125-200, 125-175, 125-150, 150-200, 150-175, 175-200, and so forth. The spacer may be about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, or 220 amino acids in length. In other cases, the spacer is less than 10 amino acids or more than 200 amino acids.

[0051] In some cases, ADRs comprise one, two, three, or more costimulatory domains that enhance cytokine production from the cells that express the ADR. The costimulatory domains may be derived from the intracellular signaling domains of costimulatory proteins

including CD28, CD27, 4-1BB, OX40, ICOS, CD30, HVEM, CD40, and so forth. As an example only, when the ADR comprises 4-1BBL, the costimulatory domain of the ADR may or may not be from 4-1BB.

[0052] In some embodiments, the ADRs will comprise a transmembrane domain that may be of any kind so long as it allows the CD3 zeta component of the ADR to be located intracellularly and the extracellular domain that targets one or more compounds that are upregulated on activated T cells to be located extracellularly. In other instances, ADRs are soluble proteins that can bind to the respective ligand on activated T cells and promote cytotoxicity by crosslinking TCR (*e.g.*, ADR-CD3 T-cell engager protein). In a case wherein the extracellular domain is from a surface protein having a transmembrane domain, (CD40, for example), the ADR may comprise the transmembrane domain from that corresponding endogenous molecule. In some cases in which the ADR molecule comprises one or more costimulatory domains, the transmembrane domain (TM) may be from the same endogenous molecule that has the costimulatory domain. Examples of TMs include those from CD3, CD8 α , CD27, CD28, 4-1BB, OX40, CD4, *etc.*

[0053] In an example of a ADR polypeptide, the components may be in a particular N-terminal (N) to C-terminal (C) order. For a general ADR, the receptor may comprise one of the following (as examples only) and wherein the extracellular domain comprises the protein that selectively binds an associated protein on an activated T cell:

N-extracellular domain-signaling domain-C

N-extracellular domain-CD3zeta-C

N-extracellular domain-spacer-CD3zeta-C

N-extracellular domain-spacer-costimulatory domain-CD3zeta-C

N-extracellular domain-spacer-two costimulatory domains-CD3zeta-C

N-two extracellular domains-spacer-costimulatory domain-CD3zeta-C

N-two extracellular domains-spacer-two costimulatory domains-CD3zeta-C

[0054] In any case, the transmembrane domain may be C-terminal with respect to the spacer. A signal peptide at the N-terminus may be utilized to facilitate expression of Type II ligands (such as OX40L and 4-1BBL) on a Type I transmembrane protein backbone (such as the transmembrane domain, signaling domains, CD3 zeta).

[0055] In some cases, the ADR comprises one or more detectable markers, such as markers that are colorimetric, fluorescent, and/or radioactive, and so forth. Examples include green fluorescent protein, blue fluorescent protein, and so forth.

[0056] The ADR may be in the form of a polynucleotide or polypeptide expressed by a polynucleotide, although the ADR may be synthetically generated as a protein. Recombinant technology to produce ADR polynucleotides and polypeptides are known in the art.

[0057] In certain cases, a ADR polynucleotide is in an expression construct or is part of an expression construct present on a vector that may be a viral vector or a non-viral vector. Examples of non-viral vectors include plasmids. Examples of viral vectors include lentiviral, retroviral, adenoviral, and adeno-associated viral vectors. Any vector expressing the ADR will have appropriate element(s) to allow expression in an eukaryotic cell, including an immune cell, such as a T cell, NK cell, or NKT cell, for example. Such appropriate elements include promoters and so forth.

[0058] 4-1BB ADR (SEQ ID NO:1)

MEFGLSWLFLVAILKGVQCGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLA
GVSLTGGLSYKEDTKELVVAAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAA
GAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGA
TVLGLFRVTPEIPAGLPSRSEESKYGPPCPPCPGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
MHEALHNHYTQKSLSLSPGKKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSRVKFS
RSADAPAYQQGQNQLYNELNLGRREEYDVLDRRRGRDPGEMGGKPRRKNPQEGLYNEL
QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRTSAAAGG
GGSGGGGSGGGGSMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYQGLTL
KFICTTGKLPVPWPTLVTTFTYGVQCFAFYPDHMKQHDFFKSAMPEGYVQERTIFFKDD
GNYKTRAIEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHKVYITADKQKNGIK
VNFKTRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSKLSKDPNEKRDHMLV
LEFVTAAGITLGMDELYK

[0059] OX40 ADR (SEQ ID NO:2)

MEFGLSWLFLVAILKGVQCQVSHRYPRIQSIKVQFTEYKKEKGFIILTSQKEDEIM
KVQNNSVIINCDGFYLISLKGYSQEVNISLHYQKDEEPLFQLKKVRSVNSLMVASLTYK

DKVYLNVTDDNTSLDDFHVNGGELILIHQNPGEFCVLESKYGPPCPPCPGQPREPQVYTL
PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKFWVLVVVGGVLACYSL
VTVAFIIFWVRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG
GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA
LHMQUALPPRTSAAAGGGGSGGGGSGGGGSMVSKGEELFTGVVPILVELDGDVNGHKFS
VSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTFTYGVQCFARYPDHMKQHDFFKS
AMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNY
NSHKVYITADKQKNGIKVNFKTRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQ
SKLSKDPNEKRDHMLLEFVTAAGITLGMDELYK

[0060] CD40L ADR (SEQ ID NO:3)

MVRLPLQCVLWGCLLTAVHPEPPTACREKQYLINSQCCSLCQPGQKLVSDCTEF
TETECLPCGESEFLDTWNRETHCHQHKYCDPNLGLRVQQKGTSETDTICTCEEGWHCTS
EACESCVLHRSCSPGFGVKQIATGVSDTICEPCPVGFFSNVSSAFEKCHPWTSCETKDLV
VQQAGTNKTDVVCQPQDRLRESKYGPPCPPCPGQPREPQVYTLPPSRDELTKNQVSLTCL
LVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGKKDPKFWVLVVVGGVLACYSLVTVAFIIFWVRSRVKFS
RSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGKPRRKNPQEGLYNE
LQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQUALPPRTSAAAG
GGGSGGGGSGGGGSMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLT
CLKFICTTGKLPVPWPTLVTTFTYGVQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKD
DGNKYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHKVYITADKQKNGI
KVNFKTRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSKLSKDPNEKRDHML
LEFVTAAGITLGMDELYK

[0061] In certain embodiments, the extracellular domain that targets activated T cells comprises an antibody or functional fragment or derivative thereof. The term "antibody," as used herein, refers to an immunoglobulin molecule that specifically binds with an antigen. Antibodies can be intact immunoglobulins derived from natural sources or from recombinant sources and can be immunoreactive portions of intact immunoglobulins. Antibodies are typically tetramers of immunoglobulin molecules. The antibodies in the present invention may exist in a variety of forms including, for example, polyclonal antibodies, monoclonal antibodies, Fv, Fab

and F(ab)₂, as well as single chain antibodies and humanized antibodies (Harlow et al., 1999, In: Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow et al., 1989, In: Antibodies: A Laboratory Manual, Cold Spring Harbor, N.Y.; Houston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; Bird et al., 1988, Science 242:423-426).

[0062] In some cases, the extracellular domain of the ADR comprises an antibody fragment. The term "antibody fragment" refers to a portion of an intact antibody and refers to the antigenic determining variable regions of an intact antibody. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')₂, and Fv fragments, linear antibodies, scFv antibodies, and multispecific antibodies formed from antibody fragments.

[0063] Synthetic antibodies may be used in the ADR. By the term "synthetic antibody" as used herein, is meant an antibody which is generated using recombinant DNA technology, such as, for example, an antibody expressed by a bacteriophage as described herein. The term should also be construed to mean an antibody that has been generated by the synthesis of a DNA molecule encoding the antibody and which DNA molecule expresses an antibody protein, or an amino acid sequence specifying the antibody, wherein the DNA or amino acid sequence has been obtained using synthetic DNA or amino acid sequence technology that is available and well known in the art.

B. Cells Expressing ADRs

[0064] Allogeneic cells used for adoptive transfer are prone to having limited efficacy because of the immune reaction by the recipient individual. Although in some cases, the cells may be modified to remove endogenous TCR (such as with CRISPR), for example to prevent graft-versus-host disease, alternatively one can utilize virus-specific T cells (intact or CAR/TCR-modified) to retain anti-viral activity, which is useful in certain pathologic conditions. Although such VSTs have very limited graft-versus-host activity because their TCRs are more restricted to viral antigens, they are still susceptible to deleterious reaction by the recipient.

[0065] Encompassed in the disclosure are cells that are improved for allogeneic use by being modified to express a synthetic ADR molecule. Thus, the disclosure includes cells harboring the ADR as a polynucleotide and as an expressed ADR polypeptide on the surface of the cells. In particular cases, the ADR-expressing cells are produced for the purpose of being maintained in a repository for off-the-shelf use. The cells may be housed in a repository already being configured to express an ADR, or they may be housed in a bank and configured to express

an ADR following retrieval from the repository. Certain cells, such as bacterial cells, may be utilized to generate the ADR molecules, whereas other cells, such as eukaryotic cells, harboring the ADR may be used for methods of the disclosure including targeting activated T cells. As shown herein, ADR-expressing immune cells selectively eliminate activated T cells and ADR-expressing immune cells are protected against cytotoxicity by alloreactive T cells.

[0066] Cells that express the ADR molecule may be of any kind, but in specific embodiments they are immune cells, for example immune effector cells, such as T cells, NK cells, NKT cells, or cell lines derived from the said lineages or engineered to have cytotoxic activity that have been modified to express the ADR and are therefore not found in nature. Populations of the non-natural ADR-expressing cells are contemplated, including populations that are at least 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% of the population being ADR-expressing cells. The cells may be generated by standard methods of transfection or transduction of synthetic ADR polynucleotides, as an example.

[0067] In some cases, the ADR molecules that modified to express the ADR molecule may already be engineered or subsequently are engineered to have another engineered, non-natural molecule other than the ADR. For example, cells that express chimeric antigen receptors (CAR) or engineered T cell receptors (TCR) and in doing so can protect such cells against host rejection and therefore increase their therapeutic potency. Cells expressing one or more CARs and/or one or more TCRs may be engineered to express one or more ADRs, or cells that express one or more ADRs may be engineered to express one or more CARs and/or one or more TCRs. Thus, in some cases a ADR is expressed on a different vector than a CAR and/or TCR, yet in other cases a ADR molecule is expressed on the same vector as a CAR and/or TCR. In cases wherein a ADR is expressed on the same vector as a CAR (as an example), the ADR and CAR expression may be directed from the same or different regulatory elements. In any case, the ADR and CAR may be expressed as a single polypeptide with a cleavable element between them, such as 2A.

[0068] In cases wherein ADR-expressing cells also express a CAR or TCR, the CAR or TCR may target any particular antigen. In cases wherein CARs are employed, the CARs may be first generation, second generation, third generation and so on. The CAR may be bispecific, in specific cases.

[0069] In some cases, cells expressing the ADR molecules are engineered, such as engineered to lack expression of one or more endogenous molecules. In specific cases the cells are engineered to lack expression of one or more endogenous genes that would facilitate fratricide of the cells otherwise. In specific cases the cells expressing the ADR molecules are engineered to lack expression of 4-1BB or OX40, for example. Engineering of the cells may occur by CRISPR/Cas9, merely as an example.

II. Methods of Using Auto/Allo-Immune Defense Receptors

[0070] Embodiments of the disclosure include methods of providing an effective amount of ADR-expressing cells to an individual for any purpose. Methods include providing selectively targeting activated T cells in an individual for any purpose. The activated T cells are targeted by exposing activated T cells to an effective amount of ADR-expressing immune cells, such as ADR-expressing T cells. Such exposure may have one or more resultant applications, for example.

[0071] In some embodiments, the ADRs are used to selectively target activated immune cells other than activated T cells, such as B cells (that would be useful for controlling unwanted B-cell responses such as with lupus, rheumatoid arthritis, *etc.*), as well as targeting activation of innate immunity (such as macrophage activation syndrome, and so on). In other embodiments, the ADRs are utilized to specifically target malignant cells expressing their corresponding target, including 4-1BB or OX40 or CD40L, for example.

[0072] The regimen for providing to an individual an effective amount of ADR-expressing cells may be known or determined by an individual or individuals delivering the cells for therapy or prevention, or regardless of the method of use. In preventative cases, for example, the cells may be delivered prior to detection of one or more symptoms, or the cells may be delivered following detection of one or more symptoms but before further symptom(s) develop and/or worsen. For treatment cases, the individual may be provided the effective amount of cells after one, two, or more symptoms develop and may be after clinical diagnosis.

[0073] In specific aspects of the methods, the individual may be given a single dose of a therapeutically effective amount of cells, or the individual may be given multiple deliveries of the therapeutically effective amount of the cells, such as multiple deliveries separated by 1, 2, 3, 4, 5, 6, 7 days, or 1, 2, 3, or 4 weeks, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or 1, 2, 3, 4,

5, or more years, or any range there between, for example. The time between doses may vary in a single regimen.

[0074] The administration of the ADR-expressing cells may be *via* any suitable route to the individual, including locally or systemically. In specific embodiments the ADR-expressing cells are delivered intravenously, orally, rectally, topically, intramuscularly, by infusion, enterically, nasally, by inhalation, sublingually, buccally, transdermally, subcutaneously, and so forth. The cells may or may not be delivered as a bolus. With multiple administrations, the cells may or may not be provided to the individual in different delivery routes. When the cells are delivered to an individual, they may be delivered in a pharmaceutically acceptable carrier or excipient. Particular examples of doses for ADR-expressing cells include 10^4 cells/m², 10^5 cells/m², 10^6 cells/m², 10^7 cells/m², 10^8 cells/m², 10^9 cells/m², 10^{10} cells/m², 10^{11} cells/m², or 10^{12} cells/m² and ranges there between.

A. Use for Off-the-Shelf Embodiments

[0075] The disclosure encompasses cells for adoptive transfer that are able to be utilized off-the-shelf, including able to be obtained from a repository for the purpose of use in an individual other than the individual from which the cells were originally obtained. The cells may already express the ADR prior to being deposited in the repository, or the cells may be modified afterwards to express the ADR. The cells may be any kind of immune effector cells for adoptive transfer. The cells prior to deposit in the repository or after obtaining them from the repository may be modified to express a tumor-specific receptor, for example (*e.g.*, a CAR or a TCR).

[0076] As shown elsewhere herein, cells expressing ADRs selectively target activated T- and NK-cells while sparing resting subsets. The ADRs protect allogeneic T cells from immune rejection mediated by T- and/or NK-cells *in vitro* in addition to protecting T-cells from immune rejection *in vivo*. The ADRs in doing so do not interfere with the function of an engineered anti-tumor receptor (CAR, as an example) as T-cells co-expressing ADR and CAR can efficiently eliminate both tumor and activated T cells *in vitro*. The disclosure further provides anti-cancer activity *in vivo* in a mouse model using “off-the-shelf” T cells co-expressing CAR and ADR while retaining resistance to immune rejection from allogeneic T-cells present in the same mice. As an example, in FIG. 14 it is shown that 4-1BB ADR is effective against 4-1BB+ tumor cells such that ADR can be used as a therapeutic modality against 4-1BB-expressing malignancies.

[0077] In particular embodiments, “off-the-shelf” therapeutic cells express ADR to resist immune rejection and either retain endogenous TCR specificity (*e.g.*, to viral or tumor antigens) or have endogenous TCRs replaced with engineered anti-tumor receptors, such as one or more CARs and/or one or more recombinant TCRs.

[0078] In specific embodiments, off-the-shelf cells are housed in a repository and may be modified for a specific purpose either before or after deposit in the repository. For example, ADR-expressing T cells may be housed in a repository and ready for use, such as after a tissue or organ transplant, to prevent graft rejection. ADR-expressing T cells may be housed in a repository and may be selected or engineered with a native or transgenic TCR, for example against viral infection or cancer. ADR-expressing T cells may be housed in a repository and may be transduced with a CAR directed to cancer or pathogenic infection. ADR-expressing cells may be housed in a repository and may be transduced with one or more CARs and/or one or more TCRs directed to specific cancer-associated antigens or neoantigens expressed by the patient's specific tumor.

[0079] In some cases, banked allogeneic cells are utilized for prevention of rejection of solid organ grafts by destroying rejecting host immune cells, particularly in cases when the ADR-expressing cells were not themselves alloreactive.

[0080] Although the cells that are housed in the repository may be allogeneic with respect to a recipient individual, in alternative embodiments the cells housed in the repository are autologous with respect to a recipient individual. For example, an individual with cancer may have ADR-expressing T cells deposited in a repository for subsequent use, such as in the event that the cancer comes out of remission. In other cases, autologous ADR-expressing cells are housed in a repository for treatment of autoimmune disorders.

B. Use in Autoimmune Disorders

[0081] Unwanted activation of endogenous autoreactive T cells in an individual can lead to devastating autoimmune diseases for the individual, such as diabetes mellitus, autoimmune colitis, and multiple sclerosis. In particular embodiments, one or more autoimmune disorders are prevented or treated using ADR-expressing immune cells in the individual that impacts the autoimmune disorder (or its potential development) by inhibiting endogenous autoreactive T cells in the individual. In specific embodiments, such use of the ADR-expressing cells spares resting non-pathogenic naïve and memory T cells in the individual. Thus, certain methods of the

disclosure utilize particular cells modified to express ADRs that are provided to an individual in a sufficient amount to target activated T cells, including pathogenic T cells, thereby initiating destruction of the activated T cells.

[0082] *In vivo* activation of T cells with unwanted specificity may cause pathogenicity, and in particular embodiments cells expressing one or more ADRs target the activated T cells. In some cases, ADR-expressing cells target pathogenic cells that are a subset of activated T cells.

[0083] In particular cases, ADR-expressing T cells can be used to prevent or reverse life-threatening and debilitating conditions driven by activated T cells (organ rejection, graft-versus-host disease, type I diabetes, multiple sclerosis, autoimmune colitis, lupus, rheumatoid arthritis, as examples) using adoptive T-cell transfer with the ADR-expressing T cells.

[0084] In some cases, the ADR-expressing T cells also comprise one or more compositions other than the ADR that facilitate treatment or prevention of one or more autoimmune disorders.

[0085] In some cases, an individual being provided the ADR-expressing T cells is given one or more additional therapies to prevent or treat one or more autoimmune disorders. The individual may or may not be given one or more immunosuppressive drugs, for example, such as glucocorticoids, cytostatics, antibodies, and/or drugs acting on immunophilins. In addition or alternatively, the individual may be given one or more appropriate vaccines.

[0086] In some cases, an individual is at risk for an autoimmune disorder and is provided an effective amount of ADR-expressing cells to prevent onset of the autoimmune disorder or to delay onset and/or lessen one or more symptoms, including in severity and/or duration, for example. An individual at risk for an autoimmune disorder, for example, is one having a personal or family history, being a female of certain ethnicity, and so forth. The individual may have one autoimmune disorder and desires to prevent or reduce the severity and/or duration or delay the onset of another autoimmune disorder(s), in some cases.

[0087] Examples of autoimmune disorders that may be prevented or treated with ADR-expressing cells include at least the following: Achalasia; Addison's disease; Adult Still's disease; Agammaglobulinemia; Alopecia areata; Amyloidosis; Ankylosing spondylitis; Anti-GBM/Anti-TBM nephritis; Antiphospholipid syndrome; Autoimmune angioedema; Autoimmune dysautonomia; Autoimmune encephalomyelitis; Autoimmune hepatitis; Autoimmune inner ear

disease (AIED); Autoimmune myocarditis; Autoimmune oophoritis; Autoimmune orchitis; Autoimmune pancreatitis; Autoimmune retinopathy; Autoimmune urticarial; Axonal & neuronal neuropathy (AMAN); Baló disease; Behcet's disease; Benign mucosal pemphigoid; Bullous pemphigoid; Castleman disease (CD); Celiac disease; Chagas disease; Chronic inflammatory demyelinating polyneuropathy (CIDP); Chronic recurrent multifocal osteomyelitis (CRMO); Churg-Strauss Syndrome (CSS) or Eosinophilic Granulomatosis (EGPA); Cicatricial pemphigoid; Cogan's syndrome; Cold agglutinin disease; Congenital heart block; Coxsackie myocarditis; CREST syndrome; Crohn's disease; Dermatitis herpetiformis; Dermatomyositis; Devic's disease (neuromyelitis optica); Discoid lupus; Dressler's syndrome; Endometriosis; Eosinophilic esophagitis (EoE); Eosinophilic fasciitis; Erythema nodosum; Essential mixed cryoglobulinemia; Evans syndrome; Fibromyalgia; Fibrosing alveolitis; Giant cell arteritis (temporal arteritis); Giant cell myocarditis; Glomerulonephritis; Goodpasture's syndrome; Granulomatosis with Polyangiitis; Graves' disease; Guillain-Barre syndrome; Hashimoto's thyroiditis; Hemolytic anemia; Henoch-Schonlein purpura (HSP); Herpes gestationis or pemphigoid gestationis (PG); Hidradenitis Suppurativa (HS) (Acne Inversa); Hypogammaglobulinemia; IgA Nephropathy; IgG4-related sclerosing disease; Immune thrombocytopenic purpura (ITP); Inclusion body myositis (IBM); Interstitial cystitis (IC); Juvenile arthritis; Juvenile diabetes (Type 1 diabetes); Juvenile myositis (JM); Kawasaki disease; Lambert-Eaton syndrome; Leukocytoclastic vasculitis; Lichen planus; Lichen sclerosus; Ligneous conjunctivitis; Linear IgA disease (LAD); Lupus; Lyme disease chronic; Meniere's disease; Microscopic polyangiitis (MPA); Mixed connective tissue disease (MCTD); Mooren's ulcer; Mucha-Habermann disease; Multifocal Motor Neuropathy (MMN) or MMNCB; Multiple sclerosis; Myasthenia gravis; Myositis; Narcolepsy; Neonatal Lupus; Neuromyelitis optica; Neutropenia; Ocular cicatricial pemphigoid; Optic neuritis; Palindromic rheumatism (PR); PANDAS; Paraneoplastic cerebellar degeneration (PCD); Paroxysmal nocturnal hemoglobinuria (PNH); Parry Romberg syndrome; Pars planitis (peripheral uveitis); Parsonage-Turner syndrome; Pemphigus; Peripheral neuropathy; Perivenous encephalomyelitis; Pernicious anemia (PA); POEMS syndrome; Polyarteritis nodosa; Polyglandular syndromes type I, II, III; Polymyalgia rheumatic; Polymyositis; Postmyocardial infarction syndrome; Postpericardiotomy syndrome; Primary biliary cirrhosis; Primary sclerosing cholangitis; Progesterone dermatitis; Psoriasis; Psoriatic arthritis; Pure red cell aplasia (PRCA); Pyoderma gangrenosum; Raynaud's phenomenon; Reactive Arthritis; Reflex sympathetic dystrophy; Relapsing polychondritis; Restless legs syndrome (RLS); Retroperitoneal fibrosis; Rheumatic fever; Rheumatoid arthritis;

Sarcoidosis; Schmidt syndrome; Scleritis; Scleroderma; Sjögren's syndrome; Sperm & testicular autoimmunity; Stiff person syndrome (SPS); Subacute bacterial endocarditis (SBE); Susac's syndrome; Sympathetic ophthalmia (SO); Takayasu's arteritis; Temporal arteritis/Giant cell arteritis; Thrombocytopenic purpura (TTP); Tolosa-Hunt syndrome (THS); Transverse myelitis; Type 1 diabetes; Ulcerative colitis (UC); Undifferentiated connective tissue disease (UCTD); Uveitis; Vasculitis; Vitiligo; Vogt-Koyanagi-Harada Disease; and Wegener's granulomatosis (or Granulomatosis with Polyangiitis (GPA)).

C. Use to Eliminate NK cells

[0088] ADR-expressing immune cells may be utilized to eliminate NK cells in cases wherein it is desirable to do so. As demonstrated herein, the presence of ADRs on certain immune cells provides a specific cytotoxic activity against NK cells that are involved with mediating rapid rejection of HLA^{low} or HLA-mismatched cells. Thus, in situations where it is desirable to maintain HLA^{low} or HLA-mismatched cells, for example to be able to utilize adoptive transfer of allogeneic cells into certain individuals, use of ADR-expressing cells avoids NK cell activation and rejection of the HLA-mismatched cells. Specifically, as shown herein, co-culture of T cells expressing ADRs leads to elimination of NK cells and thus offsets the NK cell-mediated host rejection of ADR-expressing allogeneic T cells.

D. Use to Facilitate the Engraftment of Allogeneic Cell/Tissue/Organ Transplant

[0089] In a specific aspect of avoiding activation of alloreactive T cells, one may avoid rejection of allogeneic cells, tissues, or organs in individuals receiving transplants, which is mainly mediated by a population of alloreactive T cells from the recipient. Activation of recipient's alloreactive T cells would lead to graft failure, for example, if steps were not taken to avoid such rejection. Therefore, in particular embodiments, methods of transplanting cells, tissue, or organs into an individual utilize delivery of an effective amount of ADR-expressing cells before, during, and/or after the respective transplants of cells, tissue(s), or organ(s). In some cases, the ADR-expressing cells are not themselves part of the cells, tissue(s), or organ(s) that are the subject of the transplant, whereas in other cases the ADR-expressing cells are part of the respective cells, tissue(s), or organ(s).

[0090] Tissue for transplantation may be of any kind including at least skin, cornea, bone, tendons, heart valves, veins, or arteries, for example. Organ for transplantation may be of any kind, including heart, kidneys, liver, lungs, pancreas, intestine, and thymus, for example.

[0091] In particular embodiments, ADR-expressing cells enhance allogeneic cell use in an individual as a two-pronged approach: (1) they inhibit the endogenous alloreactive T-cells in the individual; and (2) they suppress NK cell-mediated rejection in the individual. As such, the ADR molecules can enhance the persistence and activity of any type of third party-derived therapeutic cells in the individual including, for example, allogeneic therapeutic cells, including T-cells, NK cells, NK-T cells, mucosal associated invariant T cells (MAIT) and other cytotoxic cells, including those expressing engineered constructs such as chimeric antigen receptor (CAR), transgenic TCR, *etc.*

E. Use in Prophylaxis or Treatment of Graft-versus-host Disease (GvHD) during Allogeneic Cell/Tissue/Organ Transplant

[0092] In another specific aspect of avoiding activation of alloreactive T cells, one may avoid life-threatening allo-immune reactions in individuals receiving transplants of allogeneic cells, tissues, or organs. Such transplants would contain donor alloreactive T cells that would elicit development of graft-versus-host disease (GvHD), for example, if steps were not taken to avoid their activation. Therefore, in particular embodiments, methods of transplanting cells, tissue, or organs into an individual utilize delivery of an effective amount of ADR-expressing cells before, during, and/or after the respective transplants of cells, tissue(s), or organ(s). In some cases, the ADR-expressing cells are not themselves part of the cells, tissue(s), or organ(s) that are the subject of the transplant, whereas in other cases the ADR-expressing cells are part of the respective cells, tissue(s), or organ(s).

[0093] Tissue for transplantation may be of any kind including at least skin, cornea, bone, tendons, heart valves, veins, or arteries, for example. Organ for transplantation may be of any kind, including heart, kidneys, liver, lungs, pancreas, intestine, and thymus, for example.

III. Production of ADR-Expressing Cells

[0094] Cells expressing the ADR molecules may be produced in a variety of ways, all of which may be routine in the art. The production methods may include obtaining the cells to be modified to express the ADR molecule and also include generation of the ADR molecules.

A. Sources of T Cells

[0095] Prior to expansion and genetic modification of the ADR-expressing T cells of the disclosure, a source of T cells may be obtained from a subject. Such a step of obtaining may or may not be part of the method. In some cases, obtaining T cells to be modified and their

manipulation may be performed by a party other than the party that provides the ADR expressing-T cells to an individual. T cells can be obtained from a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments of the present disclosure, any number of T cell lines available in the art may be used. In certain embodiments, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as Ficoll™ separation. In one embodiment, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets, for example. In one embodiment, the cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In one embodiment, the cells are washed with phosphate buffered saline (PBS). In an alternative embodiment, the wash solution lacks calcium and may lack magnesium or may lack many if not all divalent cations. As those of ordinary skill in the art would readily appreciate a washing step may be accomplished by methods known to those in the art, such as by using a semi-automated "flow-through" centrifuge (for example, the Cobe 2991 cell processor, the Baxter CytoMate, or the Haemonetics Cell Saver 5) according to the manufacturer's instructions. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as, for example, Ca²⁺-free, Mg²⁺-free PBS, PlasmaLyte A, or other saline solution with or without buffer. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly re-suspended in culture media.

[0096] In another embodiment, T cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLL™ gradient or by counterflow centrifugal elutriation. A specific subpopulation of T cells, such as CD3⁺, CD28⁺, CD4⁺, CD8⁺, CD45RA⁺, and CD45RO⁺ T cells, can be further isolated by positive or negative selection techniques.

[0097] Enrichment of a T cell population by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method is cell sorting and/or selection *via* negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for CD4⁺ cells by negative selection, a

monoclonal antibody cocktail typically includes antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8. In certain embodiments, it may be desirable to enrich for or positively select for regulatory T cells which typically express CD4⁺, CD25⁺, CD62L^{hi}, GITR⁺, and FoxP3⁺. Alternatively, in certain embodiments, T regulatory cells are depleted by anti-CD25 conjugated beads or other similar method of selection.

[0098] For isolation of a desired population of cells by positive or negative selection, the concentration of cells and surface (e.g., particles such as beads) can be varied. In certain embodiments, it may be desirable to significantly decrease the volume in which beads and cells are mixed together (i.e., increase the concentration of cells), to ensure maximum contact of cells and beads. For example, in one embodiment, a concentration of 2 billion cells/ml is used. In one embodiment, a concentration of 1 billion cells/ml is used. In a further embodiment, greater than 100 million cells/ml is used. In a further embodiment, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet another embodiment, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further embodiments, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. In another embodiment, it may be desirable to use lower concentrations of cells. By significantly diluting the mixture of T cells and surface (e.g., particles such as beads), interactions between the particles and cells is minimized.

[0099] T cells for stimulation can also be frozen after a washing step. Washing not to be bound by theory, the freeze and subsequent thaw step provides a more uniform product by removing granulocytes and to some extent monocytes in the cell population. After the washing step that removes plasma and platelets, the cells may be suspended in a freezing solution. Many freezing solutions and parameters are known in the art. In certain embodiments, cryopreserved cells are thawed and washed as described herein and allowed to rest for one hour at room temperature prior to activation using the methods of the present invention.

[0100] Also contemplated in the context of the disclosure is the collection of blood samples or apheresis product from a subject at a time period prior to when the expanded cells as described herein might be needed. As such, the source of the cells to be expanded can be collected at any time point necessary, and desired cells, such as T cells, isolated and frozen for later use in T cell therapy for any number of diseases or conditions that would benefit from T cell therapy, such as those described herein. In one embodiment a blood sample or an apheresis is

taken from a generally healthy subject. In certain embodiments, a blood sample or an apheresis is taken from a generally healthy subject who is at risk of developing a disease, but who has not yet developed a disease, and the cells of interest are isolated and frozen for later use. In certain embodiments, the T cells may be expanded, frozen, and used at a later time. In certain embodiments, samples are collected from a patient shortly after diagnosis of a particular disease as described herein but prior to any treatments. In a further embodiment, the cells are isolated from a blood sample or an apheresis from a subject prior to any number of relevant treatment modalities, including but not limited to treatment with agents such as natalizumab, efalizumab, antiviral agents, chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies, cytoxan, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, and irradiation. These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin) (Liu et al., Cell 66:807-815, 1991; Henderson et al., Immun. 73:316-321, 1991; Bierer et al., Curr. Opin. Immun. 5:763-773, 1993). In a further embodiment, the cells are isolated for a patient and frozen for later use in conjunction with (e.g., before, simultaneously or following) bone marrow or stem cell transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAMPATH. In another embodiment, the cells are isolated prior to and can be frozen for later use for treatment following B-cell ablative therapy such as agents that react with CD20, e.g., Rituxan.

B. Activation and Expansion of T Cells

[0101] Whether prior to or after genetic modification of the T cells to express the ADR, the T cells can be activated and expanded generally using methods as described, for example, in U.S. Pat. Nos. 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005. Generally, the T cells of the disclosure are expanded by contact with a surface having attached thereto an agent that stimulates a CD3/TCR complex associated signal and a ligand that stimulates a co-stimulatory molecule on the surface of the T cells. Such processes are known in the art. In other instances, T cells can be modified to express ADR without prior activation.

C. Generation of ADR Molecules

[0102] Turning generally to polynucleotides that encode the ADR, the nucleic acid sequences coding for the ADR molecules can be obtained using recombinant methods known in the art, such as, for example by screening libraries from cells expressing the gene, by deriving the gene from a vector known to include the same, or by isolating directly from cells and tissues containing the same, using standard techniques. Alternatively, the ADR polynucleotide of interest can be produced synthetically, rather than cloned.

[0103] In brief summary, the expression of synthetic polynucleotides encoding ADRs is typically achieved by operably linking a nucleic acid encoding the ADR polypeptide or portions thereof to a promoter, and incorporating the construct into an expression vector. The vectors can be suitable for replication and integration eukaryotes. Typical cloning vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the desired nucleic acid sequence.

[0104] The ADR polynucleotide can be cloned into a number of types of vectors. For example, the nucleic acid can be cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors.

[0105] Further, the expression vector may be provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook et al. (2001, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers, (e.g., WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

[0106] A number of viral-based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. A selected gene can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either in vivo or ex vivo. A number of retroviral systems are known in the art. In

some embodiments, adenovirus vectors are used. A number of adenovirus vectors are known in the art. In one embodiment, lentivirus vectors are used.

[0107] Additional promoter elements, e.g., enhancers, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of the start site, although a number of promoters have recently been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, it appears that individual elements can function either cooperatively or independently to activate transcription.

[0108] One example of a suitable promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. Another example of a suitable promoter is Elongation Growth Factor-1alpha (EF-1alpha). However, other constitutive promoter sequences may also be used, including, but not limited to the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter. Further, the invention should not be limited to the use of constitutive promoters. Inducible promoters are also contemplated as part of the invention. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothionein promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.

[0109] In order to assess the expression of an ADR polypeptide or portions thereof, the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the

population of cells sought to be transfected or infected through viral vectors. In other aspects, the selectable marker may be carried on a separate piece of DNA and used in a co-transfection procedure. Both selectable markers and reporter genes may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers include, for example, antibiotic-resistance genes, such as neo and the like.

[0110] Reporter genes are used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a polypeptide whose expression is manifested by some easily detectable property, e.g., enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (*e.g.*, Ui-Tei et al., 2000 FEBS Letters 479: 79-82). Suitable expression systems are well known and may be prepared using known techniques or obtained commercially. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription.

[0111] Methods of introducing and expressing ADR polynucleotides into a cell are known in the art. In the context of an expression vector, the vector can be readily introduced into a host cell, e.g., mammalian, bacterial, yeast, or insect cell by any method in the art. For example, the expression vector can be transferred into a host cell by physical, chemical, or biological means.

[0112] Physical methods for introducing a ADR polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example, Sambrook et al. (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York). One method for the introduction of a polynucleotide into a host cell is calcium phosphate transfection.

[0113] Biological methods for introducing a ADR polynucleotide of interest into a host cell include the use of DNA and RNA vectors. Viral vectors, and especially retroviral vectors,

have become the most widely used method for inserting genes into mammalian, e.g., human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus I, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

[0114] Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (e.g., an artificial membrane vesicle).

[0115] In the case where a non-viral delivery system is utilized, an exemplary delivery vehicle is a liposome. The use of lipid formulations is contemplated for the introduction of the nucleic acids into a host cell (*in vitro*, *ex vivo* or *in vivo*). In another aspect, the nucleic acid may be associated with a lipid. The nucleic acid associated with a lipid may be encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the oligonucleotide, entrapped in a liposome, complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid. Lipid, lipid/DNA or lipid/expression vector associated compositions are not limited to any particular structure in solution. For example, they may be present in a bilayer structure, as micelles, or with a "collapsed" structure. They may also simply be interspersed in a solution, possibly forming aggregates that are not uniform in size or shape. Lipids are fatty substances which may be naturally occurring or synthetic lipids. For example, lipids include the fatty droplets that naturally occur in the cytoplasm as well as the class of compounds which contain long-chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes.

[0116] Lipids suitable for use can be obtained from commercial sources. For example, dimyristyl phosphatidylcholine ("DMPC") can be obtained from Sigma, St. Louis, Mo.; dicetyl phosphate ("DCP") can be obtained from K & K Laboratories (Plainview, N.Y.); cholesterol ("Choi") can be obtained from Calbiochem-Behring; dimyristyl phosphatidylglycerol ("DMPG") and other lipids may be obtained from Avanti Polar Lipids, Inc. (Birmingham, Ala.). Stock

solutions of lipids in chloroform or chloroform/methanol can be stored at about -20.degree. C. Chloroform is used as the only solvent since it is more readily evaporated than methanol. "Liposome" is a generic term encompassing a variety of single and multilamellar lipid vehicles formed by the generation of enclosed lipid bilayers or aggregates. Liposomes can be characterized as having vesicular structures with a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh et al., 1991 Glycobiology 5: 505-10). However, compositions that have different structures in solution than the normal vesicular structure are also encompassed. For example, the lipids may assume a micellar structure or merely exist as nonuniform aggregates of lipid molecules. Also contemplated are lipofectamine-nucleic acid complexes.

[0117] In some instances, the ADR molecules may be integrated into an endogenous nucleic acid of the cells. One may have a target site for homologous recombination, where it is desired that a construct be integrated at a particular locus. For example,) can knock-out an endogenous gene and replace it (at the same locus or elsewhere) with the gene encoded for by the construct using materials and methods as are known in the art for homologous recombination. For homologous recombination, one may use either OMEGA or O-vectors. CRISPR/Cas9, zinc finger nucleases, TALE nucleases, meganucleases, and other site directed nucleases may be used to target and cleave a specific site in the genome to promote homologous recombination.

[0118] The exemplary T cells that have been engineered to include the ADR-expressing construct(s) may be grown in culture under selective conditions and cells that are selected as having the construct may then be expanded and further analyzed, using, for example; the polymerase chain reaction for determining the presence of the construct in the host cells. Once the engineered host cells have been identified, they may then be used as planned, *e.g.* expanded in culture or introduced into a host organism.

[0119] Depending upon the nature of the cells, the cells may be introduced into a host organism, *e.g.* a mammal, in a wide variety of ways. The cells may be introduced at the site of the tumor, in specific embodiments, although in alternative embodiments the cells home to the

cancer or are modified to home to the infected tissue. The number of cells that are employed will depend upon a number of circumstances, the purpose for the introduction, the lifetime of the cells, the protocol to be used, for example, the number of administrations, the ability of the cells to multiply, the stability of the recombinant construct, and the like. The cells may be applied as a dispersion, generally being injected at or near the site of interest. The cells may be in a physiologically-acceptable medium.

[0120] The DNA introduction need not result in integration in every case. In some situations, transient maintenance of the DNA introduced may be sufficient. In this way, one could have a short term effect, where cells could be introduced into the host and then turned on after a predetermined time, for example, after the cells have been able to home to a particular site.

EXAMPLES

[0121] The following examples are presented in order to more fully illustrate particular embodiments of the disclosure. They should in no way, however, be construed as limiting the broad scope of the disclosure.

EXAMPLE 1

AUTO/ALLO-IMMUNE DEFENSE RECEPTORS FOR THE SELECTIVE TARGETING OF PATHOGENIC T CELLS

[0122] Disclosed herein is a novel approach to specifically target pathogenic T cells using auto/allo-immune defense receptors (ADRs) expressed on normal T cells. ADR-expressing T cells find and eliminate only activated T cells and spare resting non-pathogenic naïve and memory T cells, which constitute the majority of circulating lymphocytes

[0123] The concept of the ADR-mediated targeting is based on the observation that within 24h of activation, T cells transiently upregulate costimulatory genes 4-1BB, OX40, and/or CD40L on their cell surface. The expression of 4-1BB, OX40, and/or CD40L is only maintained when the T-cells are actively cytotoxic and is gradually downregulated within 4-5 days, when TCR signaling has stopped. Notably, activated CD8⁺ T cells showed higher magnitude of 4-1BB expression whereas CD4⁺ T cells preferentially expressed OX40 and/or CD40L. Apart from activated T cells, ADR ligands may only be expressed on activated NK cells and on some other

non-critical and replenishable subsets of cells. The pattern of expression of 4-1BB, OX40, and CD40L makes these genes attractive targets to target activated cells with high specificity while avoiding permanently damaging critical immune and non-immune tissues.

[0124] To explore the feasibility of targeting these activated T cells, auto/allo-immune defense receptors (ADRs) were designed to comprise of a 4-1BB- or OX40-specific ligand, or a CD40L-specific receptor directly connected *via* a spacer to a CD3 ζ chain encoded in a gammaretroviral vector SFG. The spacer region was incorporated a) to enable integration of type II proteins 4-1BBL and OX40L into the Type I backbone of the ADR and b) to facilitate detection of ADR on the cell surface by FACS staining. Transduction of T cells with this construct efficiently forced ADR expression on the cell surface. These ADR T cells had potent and robust cytotoxicity against 4-1BB-, OX40-, and CD40L-expressing cells, eliminating 90-99% of the target cells within 48h. These results demonstrate the feasibility of generating functional 4-1BB-, OX40-, and CD40L-specific ADR T cells.

[0125] Because ADR signaling in T cells in turn upregulates 4-1BB, OX40, and CD40L, thus promoting fratricide and impeding effector cell expansion, the effects of CRISPR/Cas9 genomic disruption of ADR target genes in the effector T cells were explored. The inventors had previously shown that this CRISPR/Cas9 approach can prevent fratricide of primary human T cells expressing a CD7-specific CAR. In this context, with CRISPR/Cas9 the inventors were able to knock out 4-1BB expression in ~70% of ADR T cells that consistently increased ADR T cell expansion >2-fold at 48h following coculture with 4-1BB⁺ target cells without affecting the cytotoxicity.

[0126] Next, the ability of ADR T cells was tested to selectively eliminate activated T cells. 4-1BB, OX40, and CD40L ADR T cells were co-cultured with fluorescently labeled resting or CD3/CD28 activated T cells. Residual live CD4⁺ and CD8⁺ T cells were quantified by flow cytometry with counting beads. There was no reactivity against resting autologous T cells after 72h of co-culture with T cells expressing 4-1BB-, OX40-, or CD40L-specific ADR (FIG. 2B). In contrast, co-culture of 4-1BB ADR T cells with CD3/CD28-activated T cells eliminated most CD8⁺ and some CD4⁺ T cells within 48h. Incubation with OX40 ADR T cells resulted in a reciprocal high-level depletion of activated CD4⁺ T cells and modest depletion of activated CD8⁺ T cells. CD40L ADR T cells produced moderate cytotoxic effect on activated CD4⁺ T cells yet no effect was seen on CD8⁺ T cells. The differential targeting profiles of OX40, CD40L and 4-

1BB ADR T cells against activated CD4⁺ and CD8⁺ T cells correlates with observed differences in the magnitude and kinetics of OX40, CD40L and 4-1BB expression on each T cell subset. This property of ADRs can be utilized to preferentially target either or both subsets of allo- or auto-reactive T cells according to need. Therefore, ADR expression enables T cells to specifically target activated (pathogenic) T cells but spare resting cells, suggesting their clinical use.

[0127] It was assessed whether virus-specific T cells (VST) expressing ADRs can resist allogeneic rejection in an *in vitro* mixed lymphocyte reaction (MLR) assay. CMV-specific T cells were generated from an HLA-A2-negative donor and mixed control non-transduced or ADR-transduced VST with alloreactive HLA-A2⁺ PBMC at a 1:2 cell-to-cell ratio. The inventors then cultured the cells for 12 days. At the end of co-culture, control VSTs were almost completely eliminated by HLA-A2⁺ PBMC whereas VSTs expressing either 4-1BB ADR or OX40 ADR resisted rejection. Taken together, these results demonstrate the feasibility and selectivity of targeting activated T cells using the newly developed ADR platform embodiment.

EXAMPLE 2

AUTO/ALLO-IMMUNE DEFENSE RECEPTORS FOR THE SELECTIVE TARGETING OF NK CELLS

[0128] The ADRs demonstrate a specific cytotoxic activity against NK cells, a key cell population mediating rapid rejection of HLA^{low} or HLA-mismatched cells.

[0129] NK cells are capable of recognizing HLA-mismatched cells or cells with low HLA expression, as a part of the anti-tumor and anti-viral immune surveillance. Adoptive transfer of allogeneic cells into immunoreplete patients would thus result in NK-cell activation and rejection of the HLA-mismatched cells. Here, it is shown that co-culture of T cells expressing 4-1BB- and OX40-specific auto/allo-immune defense receptors (ADRs) leads to elimination of NK cells and thus would offset the NK cell-mediated host rejection of ADR-armed allogeneic T cells. Therefore, ADRs do not only inhibit the alloreactive T-cell response but also suppress NK cell mediated rejection, further supporting the application of ADRs to enhance the persistence and activity of “off-the-shelf” therapeutic T cells.

EXAMPLE 3

ADR-EXPRESSING T CELLS ELIMINATE TARGET CELLS

[0130] ADRs can be expressed on cell surface of immune cells and promote cytotoxicity against respective targets. FIG. 1A illustrates one example of a schematic of ADR (a label such as GFP is optional). The expression of ADR on the surface of T cells was confirmed (FIG. 1B) and the cells were expanded commensurate with controls (FIG. 1C). (FIG. 1D) The ADR-expressing T cells were cytotoxicity against target cells expressing corresponding ADR ligands (FIG. 1D). FIG. 1E demonstrates expansion of wild-type vs 4-1BB KO T cells expressing 4-1BB ADR and their cytotoxicity against 4-1BB+ targets. Knocking out the ADR ligand on T cells can further enhance expansion and cytotoxicity, and co-expression of ADR and its ligand on T cells is not required for ADR-T cell expansion or function (FIG. 1E).

[0131] Selective expression of ADR ligands on activated T cells enables their selective elimination by ADR T cells. Expression of ADR ligands on resting vs activated T cells after TCR stimulation is determined (FIG. 2A- FIG. 2C). There was no cytotoxicity of ADR T cells against resting CD4+ and CD8+ T cells (FIG. 2D), yet there was elimination of activated CD4+ and CD8+ T cells by ADR T cells after a 48h co-culture (FIG. 2E).

[0132] As one example, expression of 4-1BB ADR protects T cells from immune rejection in an MLR model. Representative dot plots showing TCRKO T cells co-expressing ADR are protected from rejection after co-culture with allogeneic PBMC at a 1:10 ADR T:PBMC ratio (FIG. 3A). Absolute counts of donor T cells and allogeneic T cells in the PBMC during co-culture (FIG. 3B- FIG. 3C) are the same for virus-specific ADR T cells (FIG. 3D- FIG. 3F).

[0133] Expression of ADR protects allogeneic virus-specific T cells from immune rejection in a mixed lymphocyte reaction *in vitro*. Representative dot plots showing ADR VST are protected from immune rejection by recipient allogeneic PBMC (FIG. 4A). The absolute counts of recipient T cells and donor VST at various time points during MLR are provided in FIG. 4B and FIG. 4C.

[0134] In FIG. 5, ADR VSTs retain anti-viral function. ADR VSTs were co-cultured with viral pepmix-pulsed monocytes, and monocyte counts indicated that they eliminated viral infected cells equally well compared to unmodified VSTs.

[0135] Activated NK cells upregulate ADR ligands and can be selectively targeted by ADR T cells. Expression of 4-1BB on resting vs activated NK cells is confirmed (FIG. 6A- FIG. 6B). Residual counts of resting vs activated NK cells after 24hr co-culture with 4-1BB ADR T cells are determined (FIG. 6C). In FIG. 6D, ADR T cells lacking MHC are protected from immune rejection by allogeneic PBMC by controlling the expansion of NK cells. Absolute counts of donor T cells and allogeneic NK cells during co-culture are determined in FIG. 6E. ADR T cells lacking MHC resist immune rejection by NK cells upon 48h co-culture at a 1:1 E:T ratio (FIG. 6F). ADR T cells control the expansion of alloreactive NK cells during MLR with PBMC (FIG. 6G), with absolute counts of NK cells plotted in FIG. 6H.

[0136] ADR expression protects allogeneic T cells from immune rejection *in vivo*. In FIG. 7A, one example is shown of a mouse model of immune rejection where mice were given T cells from an HLA-A2+ donor after a sublethal irradiation, followed by administration of allogeneic HLA-A2- T cells 4 days later. Control T cells from the HLA-A2- donor were rejected by Day 18 while ADR-expressing cells were protected (FIG. 7B). Absolute counts of T cells from HLA-A2+ and HLA-A2- donors at various time points were determined (FIG. 7C). A modified *in vivo* model in FIG. 7D depicts where, instead of allogeneic T cells mice, received whole PBMC (containing both T- and NK-cells) from donor 1. Representative flow plots in FIG. 7E show that ADR T cells were protected from immune rejection and also protected mice from rapid onset of fatal GvHD.

[0137] Coexpression of CAR and ADR preserves functions of both receptors. FIG. 8A illustrates an example of a representation of an immune cell co-expressing ADR and a CAR. Coexpression of a CAR and an ADR on the cell surface were confirmed (FIG. 8B). In FIG. 8C, cytotoxicity is shown of CAR-ADR T cells against NALM-6 (A CD19+ CAR target), as one example of a target. Cytotoxicity of the CAR-ADR T cells against activated T cells (ADR target) were also determined in FIG. 8D. Cytotoxic activity of CAR-ADR T cells against both targets upon simultaneous co-culture with both cell targets is demonstrated in FIG. 8E.

[0138] CAR-ADR T cells are protected from immune rejection and exert potent anti-tumor activity. An example of a mouse model and a regimen is depicted in FIG. 9A. Mice received allogeneic T cells from Donor 1 and b2mKO NALM6 24hr apart, followed by a single dose of CAR-ADR T cells from Donor 2, as one example of a regimen. Kinetics of T cells from Donor 2 in peripheral blood are provided in FIG. 9B, and kinetics of Donor 1 T cells in the

experimental groups are provided in FIG. 9C. FIG. 9D shows leukemia burden in the mice, with determination of overall survival of the mice (FIG. 9E).

[0139] FIGS. 13A-13C. CAR-ADR T cells are protected from immune rejection and exert potent anti-tumor activity in a solid tumor model. Schematic of an example of a mouse model and treatment is shown in FIG. 10A, wherein mice received allogeneic T cells from Donor 1 and b2mKO neuroblastoma cell line CHLA255 24hr apart, followed by a single dose of CAR-ADR T cells from Donor 2. Donor 2 GD2 CAR T cells were rejected by D18, whereas CAR-ADR T cells resisted allogeneic rejection and persisted in peripheral blood (FIG. 10B). Tumor burden in mice is shown in FIG. 13C, where * indicates xenogeneic-GvHD associated deaths in ATC+GD2 CAR T group.

[0140] TCR-knockout CAR-ADR T cells are protected from immune rejection and exert potent anti-tumor activity. Schematic of the mouse model is provided in which mice received allogeneic T cells from Donor 1 and b2mKO NALM6 24hr apart, followed by a single dose of TCR-edited CAR-ADR T cells from Donor 2 (FIG. 11A). Kinetics of T cells from Donor 2 in peripheral blood are provided (FIG. 11B). Kinetics of Donor 1 T cells in the experimental groups are shown (FIG. 11C). Leukemia burden in mice (FIG. 11D) and overall survival of mice are provided (FIG. 11E).

[0141] ADR T cells protect mice against fatal xenogeneic GvHD. Schematic of a model is provided in FIG. 12A, and expansion of FFLuc-labeled ADR T cells *in vivo* is demonstrated (FIG. 12B). Kinetics of weight gain/loss in mice were determined (FIG. 12C). Overall survival of mice is depicted (FIG. 12D).

[0142] Second generation ADR with CD28 intracellular signaling domain ("ADR.28zeta") (as one example) were utilized. One example of a structure of ADR.28zeta is depicted (FIG. 13A). *In vitro* cytotoxicity was determined of ADR.28zeta against target-expressing cells ((FIG. 13B and FIG. 13C). ADR.28zeta protected mice from xeno-GvHD lines (FIG. 13B). Schematic of the model (FIG. 13D) is shown. Expansion of FFLuc-labeled ADR.28zeta T cells *in vivo* was confirmed (FIG. 13E) Kinetics of weight gain/loss in mice (FIG. 13F), and the overall survival of mice was determined (FIG. 13G).

[0143] Although the present disclosure and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein

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

CLAIMS

What is claimed is:

1. An isolated polynucleotide, comprising sequence encoding a polypeptide, wherein said polypeptide comprises:
 - (1) one or more of an OX40-specific ligand, a 4-1BB-specific ligand, CD40L-specific ligand, or functional derivatives thereof; that is operably linked to
 - (2) a signaling domain promoting T-cell activation.
2. The polynucleotide of claim 1, wherein the polypeptide comprises an OX40-specific ligand.
3. The polynucleotide of claim 1, wherein the polypeptide comprises a 4-1BB-specific ligand.
4. The polynucleotide of claim 1, wherein the polypeptide comprises CD40L-specific ligand.
5. The polynucleotide of any one of claims 1-2, wherein the OX40-specific ligand is OX40L, an antibody that targets OX40, an OX40L-Fc fusion, or a combination thereof.
6. The polynucleotide of any one of claims 1 and 3, wherein the 4-1BB-specific ligand is 4-1BBL, an antibody that targets 4-1BB, a 4-1BBL-Fc fusion, or a combination thereof.
7. The polynucleotide of any one of claims 1 and 4, wherein the CD40L-specific ligand is CD40, an antibody that targets CD40L, a CD40-Fc fusion, or any other engineered protein capable of specific binding to CD40L.
8. The polynucleotide of any one of claims 1-7, wherein the polypeptide further comprises 1, 2, or more costimulatory domains.
9. The polynucleotide of any one of claims 1-8, wherein the polynucleotide further comprises sequence that encodes a spacer between (1) and (2).

10. The polynucleotide of claim 9, wherein the spacer is between 10 and 220 amino acids in length.
11. The polynucleotide of claim 10, wherein the spacer has sequence that facilitates surface detection with an antibody.
12. The polynucleotide of claim 11, wherein the spacer is detectable with an anti-Fc Ab.
13. The polynucleotide of claim 12, wherein the spacer comprises IgG Fc portion.
14. The polynucleotide of any one of claims 1-13, wherein the polynucleotide further encodes a chimeric antigen receptor, a T-cell receptor, or both.
15. The polynucleotide of claim 14, wherein there is a 2A element or IRES element on the polynucleotide that encodes the polypeptide of claim 1 and the polynucleotide that encodes the chimeric antigen receptor, a T-cell receptor, or both.
16. The polynucleotide of claim 15, wherein the chimeric antigen receptor comprises 1, 2, or more costimulatory domains.
17. The polynucleotide of any one of claims 1-16, wherein the polynucleotide is present on a vector.
18. The polynucleotide of claim 17, wherein the vector is a viral vector or non-viral vector.
19. The polynucleotide of claim 18, wherein the viral vector is a retroviral vector, lentiviral vector, adenoviral vector, or adeno-associated viral vector.
20. The polynucleotide of any one of claims 1-19, wherein the polynucleotide is present in a cell.
21. The polynucleotide of claim 20, wherein the cell is a eukaryotic cell or a bacterial cell.
22. The polynucleotide of claim 20 or 21, wherein the cell is an immune cell.
23. The polynucleotide of any one of claims 20-22, wherein the cell is engineered.
24. The polynucleotide of claim 22, wherein the immune cell is a T cell.

25. The polynucleotide of claim 24, wherein the T cell comprises one or more chimeric antigen receptors.
26. The polynucleotide of claim 25, wherein the chimeric antigen receptor comprises 1, 2, or more costimulatory domains.
27. The polynucleotide of claim 24, wherein the T cell comprises one or more engineered T cell receptors (TCRs).
28. A polypeptide expressed by a polynucleotide of any one of claims 1-27.
29. A polypeptide, comprising:
- (1) one or more of an OX40-specific ligand, a 4-1BB-specific ligand, and CD40; that is operably linked to
 - (2) a signaling domain promoting T-cell activation.
30. The polypeptide of claim 29, wherein the signaling domain promoting T-cell activation is, from CD3 zeta subunit, DAP12, an Fc receptor, or a combination thereof.
31. The polypeptide of claim 29 or 30, wherein the polypeptide further comprises 1, 2, or more costimulatory domains.
32. A chimeric receptor-expressing cell, comprising the polynucleotide of any one of claims 1-27 or the polypeptide of any one of claims 29-31.
33. The cell of claim 32, wherein the cell is an immune cell.
34. The cell of claim 32 or 33, wherein the cell is engineered.
35. The cell of claim 33, wherein the immune cell is a T cell.
36. The cell of claim 35, wherein the T cell is a CAR-transduced T cell.
37. The cell of claim 35, wherein the T cell is a T cell receptor (TCR)-transduced T cell.
38. The cell of any one of claims 32-37, wherein the cell is engineered to lack endogenous expression of one or more genes.

39. The cell of claim 38, wherein the cell is engineered to lack endogenous expression of 4-1BB, OX40 and/or CD40L.
40. The cell of claim 38 or 39, wherein the cell is engineered using CRISPR/Cas9, zinc finger nucleases, TALE nucleases, or meganucleases.
41. The cell of any one of claims 32-40, wherein the cell is housed in a cell repository.
42. A method of preparing cells for cell therapy, comprising the step of transfecting immune effector cells with the polynucleotide of any one of claims 1-27.
43. The method of claim 42, further comprising the step of depositing the cells in a cell repository.
44. The method of claim 42 or 43, further comprising the step of modifying the cells to express one or more chimeric antigen receptors and/or one or more recombinant T-cell receptors.
45. The method of any one of claims 42-44, further comprising the step of providing an effective amount of the cells to an individual in need thereof.
46. The method of claim 45, wherein the cells are allogeneic with respect to the individual.
47. A method of treating an individual for a medical condition with allogeneic therapeutic cells, comprising the step of providing to the individual a therapeutically effective amount of allogeneic cells, wherein said cells express a polypeptide comprising (1) one or more of an OX40-specific ligand, a 4-1BB-specific ligand, CD40L-specific ligand, or functional derivatives thereof; that is operably linked to (2) a signaling domain promoting T-cell activation.
48. The method of claim 47, wherein the cells are obtained from a cell repository.
49. The method of claim 47 or 48, wherein the cells express one or more chimeric antigen receptors and/or one or more recombinant T-cell receptors.
50. A method of avoiding rejection of allogeneic cells, tissue, or organs in an individual, comprising the step of delivering to the individual an effective amount of allogeneic immune cells expressing an engineered chimeric receptor that comprises an extracellular domain that targets compounds that are selectively present on activated T cells and that comprises CD3 zeta,

wherein the delivering step results in the following in the individual:

- (1) inhibition of endogenous alloreactive T cells in the individual; and/or
- (2) suppression of NK cell activation in the individual.

51. The method of claim 50, wherein the allogeneic cells are the allogeneic immune cells expressing the chimeric receptor.

52. The method of claim 50 or 51, wherein the allogeneic cells express a chimeric antigen receptor or an engineered T cell receptor.

53. The method of claim 50, wherein the allogeneic immune cells are delivered to the individual before, during, and/or after tissue and/or organ transplantation in the individual.

54. The method of any one of claims 50-53, wherein the activated T cells are pathogenic T cells.

55. A method of selectively targeting activated T cells in an individual, comprising the step of providing to the individual an effective amount of immune cells expressing an engineered chimeric receptor, said chimeric receptor comprising:

- (1) an extracellular domain that targets compounds that are selectively present on activated T cells; and
- (2) a signaling domain promoting T-cell activation.

56. The method of claim 55, wherein the signaling domain promoting T-cell activation is derived from CD3 zeta subunit, DAP12, an Fc receptor, or a combination thereof.

57. The method of claim 55 or 56, wherein the activated T cells are pathogenic T cells.

58. A method of preventing or treating a medical condition related to activated T cells in an individual, comprising the step of delivering to the individual an effective amount of immune cells expressing an engineered chimeric receptor that selectively targets said activated T cells, said chimeric receptor comprising:

(1) an extracellular domain that targets compounds that are selectively present on activated T cells; and

(2) a signaling domain promoting T-cell activation.

59. The method of claim 58, wherein the chimeric receptor further comprises 1, 2, or more costimulatory domains.

60. The method of claim 58 or 59, wherein the medical condition is an autoimmune disorder.

61. The method of any one of claims 58-60, wherein the medical condition comprises graft rejection, graft-versus-host disease, type I diabetes, multiple sclerosis, autoimmune colitis, or a combination thereof.

62. A method of avoiding NK cell-mediated host rejection of allogeneic T cells, tissues, or organs in an individual, comprising the step of providing to the individual an effective amount of immune cells expressing an engineered chimeric receptor that comprises an extracellular domain that targets compounds that are selectively present on activated T cells and that also comprises a signaling domain promoting T-cell activation.

63. The method of claim 62, wherein the immune cells expressing the engineered chimeric receptor are the allogeneic T cells.

64. The method of claim 62 or 63, wherein the immune cells express a chimeric antigen receptor or an engineered T cell receptor.

65. The method of any one of claims 62-64, wherein the amount of immune cells expressing the engineered chimeric receptor that are provided to the individual are in the range of 10^2 - 10^{12} per m^2 .

66. The method of any one of claims 47-65, wherein the cells expressing the chimeric receptor are provided to the individual systemically or locally.

67. The method of any one of claims 47-66, wherein the immune cells are T cells.

68. The method of any one of claims 47-67, wherein the immune cells are delivered to the individual once or more than once.