

MAJOR HISTOCOMPATIBILITY COMPLEX-BASED CHIMERIC RECEPTORS AND USES THEREOF FOR TREATING AUTOIMMUNE DISEASES

RELATED APPLICATION

This application claims the benefit of the filing date of U.S. Provisional Application Serial No. 62/584,449, filed on November 10, 2018. The entire contents of this referenced application are incorporated by reference herein.

BACKGROUND OF THE INVENTION

Autoimmune diseases are characterized by abnormal immune responses against self-antigens, leading to damage or disruption of tissues. Multiple sclerosis (MS) is a central nervous system autoimmune disease, in which activated autoreactive T cells invade the blood brain barrier, initiating an inflammatory response that leads to myelin destruction and axonal loss. Although the etiology of MS, the mechanisms associated with its onset and progression, and determination of its outcome remains unelucidated, all available evidence suggests that therapies specifically targeting the pathologic immune cells responsible for MS would have improved therapeutic outcomes over available therapies. Reinhard et al., *Proceedings of the National Academy of Sciences*, 101(suppl 2):14599–14606; 2004. This strategy could be extended to other immune disorders with similar mechanisms, including rheumatoid arthritis. Carol et al., *Nature Reviews Immunology*, 2(2):85–95, 2002.

The major histocompatibility complex (MHC), known as human leukocytes (HLA) in humans, is a set of cell surface proteins essential for the immune system to recognize foreign agents. MHC complexes bind to antigens derived from pathogens and display such to T cells, which are then activated, leading to elimination of cells displaying foreign antigens. MHC complexes may also display intact, and in some cases misfolded, host-derived proteins to B cells thereby inducing the autoantibody responses characteristic of autoimmune disorders. Jiang et al., *International immunology*, 25(4):235-246 (2013), and Busch et al., *The EMBO journal*, 15(2):418, (1996).

SUMMARY

In example, the disclosure features a major histocompatibility complex (MHC)-based chimeric receptor (CAR), comprising: (i) an extracellular domain of a MHC molecule conjugated to an antigenic peptide from an antigen involved in an autoimmune disease; and (ii) a cytoplasmic signaling domain, at least one co-stimulatory domain, or a combination

thereof. The MHC-based CAR may further comprises a hinge domain located between (i) and (ii). The antigenic peptide is dependent on the autoimmune disorder and may be from myelin basic protein (MBP), proteolipid protein (PLP), insulin, glutamate decarboxylase, or the additional exemplary self-antigens as described in Table 1.

In some examples, the MHC-based chimeric receptor comprises at least one co-stimulatory domain, which may be a co-stimulatory domain from 4-1BB (CD137), a co-stimulatory domain from CD28, or a combination thereof. In other examples, the MHC-based chimeric receptor as described herein may be free of a cytoplasmic signaling domain. Alternatively or in addition, the MHC-CAR comprises a cytoplasmic signaling domain of CD3 ζ .

In some examples, the MHC molecule in the MHC-CAR is a class I MHC, for example, a human class I MHC. In some instances, the extracellular domain of the chimeric receptor comprises an extracellular domain of the alpha chain of the class I MHC, which is fused to the antigenic peptide. For example, the chimeric receptor may be a fusion polypeptide comprising (i) the extracellular domain of the class I MHC molecule, and (ii) the cytoplasmic domain, the at least one co-stimulatory domain, or the combination thereof. In one example, the chimeric receptor is a fusion polypeptide, which comprises, from N-terminus to C-terminus, a signal peptide, a first peptide linker, the antigenic peptide, a second peptide linker, an extracellular domain of macroglobulin, a third peptide linker, the class I MHC molecule, a transmembrane domain, the at least one co-stimulatory domain, and CD3 ζ .

In other examples, the MHC-based chimeric receptor as described herein comprises a class II MHC (*e.g.*, a human MHC II) or a portion thereof. Such a chimeric receptor may comprise a first polypeptide, which comprises an extracellular domain of a first MHC class II, and a second polypeptide, which comprises an extracellular domain of a beta chain of a second MHC class II, and wherein the antigenic peptide is fused to either the first polypeptide or the second polypeptide, and wherein either the first polypeptide or the second polypeptide further comprises the cytoplasmic signaling domain, the at least one co-stimulatory domain, or the combination thereof. In some examples, the chimeric receptor can be a fusion polypeptide comprising (i) an extracellular domain of the alpha chain of a first MHC class II molecule, (ii) an extracellular domain of the beta chain of a second MHC class II molecule, (iii) the antigenic peptide, and (iv) the cytoplasmic signaling domain, the at least one co-stimulatory domain, or the combination thereof. In some examples the antigenic protein may not be linked to the MHC class II and may instead be expressed as a separate

fusion polypeptide with an alternative signal peptide (such as that from CD150, *i.e.*, MDPKGLLSLTFVLFLSLAFG (SEQ ID NO: 388)). In some examples, the first MHC class II is HLA-DRA*1010. Alternatively or in addition, the second MHC class II is HLA-DRB1*1501.

In another example, the present disclosure features a nucleic acid or a nucleic acid set, which collectively encodes any of the MHC-based chimeric receptors described herein. In some instances, the nucleic acid or nucleic acid set can be located in one or more vectors, for example, viral vector(s).

Further, the present disclosure provides a genetically modified immune cell (*e.g.*, a T cell), which expresses any of the MHC-based chimeric receptors described herein. In some instances, the activity of the endogenous T cell receptor (TCR) can be suppressed, which may be achieved by mutating or deleting the alpha chain of the endogenous TCR, the beta chain of the endogenous TCR, or both to disrupt surface expression of the endogenous TCR. Alternatively or in addition, the expression of the endogenous CD52 can be disrupted.

In some examples, the genetically modified immune cell as described herein may further express a suicide gene (*e.g.*, RQR8), a marker gene (*e.g.*, GFP), or both. When necessary, the immune cell can be further modified for lymph node or tertiary lymphoid organ delivery and retention. For example, the immune cell can be further engineered to overexpress VAP-1, L-selectin, CCR7, CXCR5, or a combination thereof. In some instances, the expression of endogenous sphingosine-1-phosphate receptor 1 can be disrupted in the genetically modified immune cell.

In some examples, the immune cell can be engineered to travel to the site of inflammation, for instance using a chemokine receptor such as CCR6 (*e.g.*, to the site of Th17 cells), CXCR3 or CXCR4 (*e.g.*, to the site of plasma cells), or through a membrane linked, antigen targeted antibody. Alternatively or in addition, the genetically modified immune cell may further comprise a genetic modification that results in blockade of PD-1 signaling. If needed and the disorder is especially severe the MHC-CAR cells can also be designed to remove or inactive bystander B cells (with a CD19 or CD20-CAR) or plasma cells (with a CS1-CAR and/or CS1 knockout).

In some examples, the genetically modified immune cell as described herein may be a regulatory T cell, which can be CD25⁺, and optionally CD4⁺. In some instances, the regulatory T cell can be derived from CD25⁺⁺CD45R⁺ T cells isolated from peripheral blood mononuclear cells or from cord blood. In other instance, the regulatory T cell may

comprise a transgene coding for CD25. Any of the Treg cells disclosed herein may further express a chimeric receptor specific to CD19, a chimeric receptor specific to CS-1, or both. Alternatively or in addition, the regulatory T cell may further express CCR6, CXCR5, PD-1, or a combination thereof. In some examples, the regulatory T cell may display an antibody specific to MOG.

In yet another example, the present disclosure provides a method for suppressing autoreactive immune cells in a subject having an autoimmune disease (*e.g.*, multiple sclerosis). The method may comprise administering to the subject an effective amount of genetically modified immune cells as described herein, which can be T cells.

In some examples, the genetically modified immune cells are autologous. In other examples, the genetically modified immune cells are allogeneic. Any of the genetically modified immune cells may be administered to a lymph node of the subject. In some instances, the subject is undergoing a therapy comprising an antibody specific to CD52.

In some examples, the subject is a human patient having or at risk for multiple sclerosis and the genetically modified T cells are Treg cells or cytotoxic lymphocytes (CTLs) as described herein.

In some examples, the human patient is an early-stage MS patient and the Treg cells express the MHC-CAR and have one or more of the following genetic modifications: (i) PD-L1 and/or PD-1 knockout; (ii) surface expression of CCR6 and/or CXCR5; (iii) surface display of an antibody or an antigen-binding fragment thereof that is specific to MOG; and (iv) surface expression of a chimeric receptor targeting CD19. In some examples the patient may first, simultaneously, or alternatively be treated with cytotoxic CTLs with modifications of the same type.

In some examples, the human patient has relapsing-remitting MS or early-stage progressive MS and the Treg cells express the MHC-CAR and have one or more of the following modifications: (i) surface display of an antibody or antigen binding fragment that is specific to MOG; and (ii) surface expression of CCR6. In some examples the patient may first, simultaneously, or alternatively be treated with cytotoxic CTLs with modifications of the same type.

In some examples, the human patient has relapsing-remitting MS or early-stage progressive MS and the Treg cells express the MHC-CAR and have one or more of the following modifications: (i) surface expression of a chimeric receptor targeting CD19; and (ii) surface expression of CXCR5. In some examples the patient may first, simultaneously, or

alternatively be treated with CTLs with the same modifications.

In some examples, the human patient has MS in chronic progressive form and the Treg cells express the MHC-CAR and have one or more of the following modifications: (i) surface expression of a chimeric receptor targeting CS-1; and (ii) surface expression of an agent CXCR4, CCR6, and/or CXCR5. In some examples the patient may first, simultaneously, or alternatively be treated with CTLs with the same modifications.

In some examples, the subject is a human patient having or at risk for systemic lupus erythematosus, rheumatoid arthritis, juvenile idiopathic arthritis (also known as juvenile idiopathic arthritis), Sjögren's syndrome, systemic sclerosis, ankylosing spondylitis, Type 1 diabetes, autoimmune thyroid diseases (Grave's and Hashimoto's), multiple sclerosis myasthenia gravis, inflammatory bowel disease (Crohn's or ulcerative colitis), Psoriasis, or a diseases mentioned in Table 1 and the genetically modified T cells are Treg cells and/or CTLs as described herein.

In some examples, the human patient is an early-stage patient of any of the autoimmune disorders described herein (e.g., those listed in Table 1) and the Treg cells express the MHC-CAR and have one or more of the following genetic modifications: (i) PD-L1 and/or PD-1 knockout; (ii) surface expression of CCR6 and/or CXCR5; (iii) surface display of an antibody or an antigen-binding fragment thereof that is specific to a relevant protein described as an autoantigen in Table 1 for that autoimmune disorder; and (iv) surface expression of a chimeric receptor targeting CD19. In some examples the patient may first, simultaneously, or alternatively be treated with CTLs with the same modifications.

In some examples, the human patient has moderately severe disease state of any of the autoimmune disorders as described herein (e.g., those listed in Table 1) and the Treg cells express the MHC-CAR and have one or more of the following modifications: (i) surface display of an antibody or antigen binding fragment that is specific to a relevant protein described as an autoantigen in Table 1 for that autoimmune disorder; and (ii) surface expression of CCR6. In some examples the patient may first, simultaneously, or alternatively be treated with CTLs with the same modifications.

In some examples, the human patient has moderately severe disease state of any of the autoimmune disorders as described herein (e.g., those listed in Table 1) and the Treg cells express the MHC-CAR and have one or more of the following modifications: (i) surface expression of a chimeric receptor targeting CD19; and (ii) surface expression of CXCR5. In some examples the patient may first, simultaneously, or alternatively be treated with CTLs

with the same modifications.

In some examples, the human patient has severe disease state of any of the autoimmune disorders described herein (e.g., those listed in Table 1) and the Treg cells express the MHC-CAR and have one or more of the following modifications: (i) surface expression of a chimeric receptor targeting CS-1; and (ii) surface expression of an agent targeting CXCR4, CCR6, and/or CXCR5. In some examples the patient may first, simultaneously, or alternatively be treated with CTLs with the same modifications.

Also within the scope of the present disclosure are pharmaceutical compositions for use in treating an autoimmune disease, the composition comprising genetically modified immune cells expression MHC-CAR as described herein such as Treg cells and a pharmaceutically acceptable carrier, and uses of such genetically modified immune cells for manufacturing a medicament for use in treating the target autoimmune disease.

The details of one or more examples of the disclosure are set forth in the description below. Other features or advantages of the present disclosure will be apparent from the following drawings and detailed description of several examples, and also from the appended claims.

In one aspect, the present disclosure refers to a major histocompatibility complex (MHC)-based chimeric receptor, comprising: (a) a first polypeptide, which comprises an extracellular domain of a first MHC class II, and (b) a second polypeptide, which comprises an extracellular domain of a beta chain of a second MHC class II, wherein an antigenic peptide is fused to either the first polypeptide or the second polypeptide, and wherein either the first polypeptide or the second polypeptide, but not both, further comprises a cytoplasmic signaling domain.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic illustration of an exemplary design of a lentiviral expression vector for expression of an antigen-specific T cell receptor (TCR). mRNA and multicistronic mRNA designs are similar.

Figure 2 is a schematic illustration of an MHC-CAR T cell, which expresses various designs of MHC-CAR as indicated in conjugation with a myelin basic protein (MBP) peptide for targeting pathologic T cells involved in multiple sclerosis (MS). Optionally, the MHC-CAR T cell may have the endogenous TCR and/or CD52 knocked out. The MHC-CAR T cell may further express RQR8 on the cell surface.

Figure 3 is a schematic illustration of exemplary designs for various MHC-CAR constructs. The exemplary MHC-CAR constructs may have two subunits: an α -chain containing a leader sequence, a DRA*1010 domain, and a cytoplasmic domain, and a β -chain that includes a leader sequence from HLA-DRB1*1501, a peptide from MBP, and a domain from HLA-DRB1*1501. The DNA used to create mRNA contains either single chains or are multicistronic and separated by orthogonal 2A sequences. RQR8 and eBFP are used for both cell control and labeling.

What Is Claimed Is:

1. A major histocompatibility complex (MHC)-based chimeric receptor, comprising:
 - (a) a first polypeptide, which comprises an extracellular domain of a first MHC class II, and
 - (b) a second polypeptide, which comprises an extracellular domain of a beta chain of a second MHC class II,

wherein an antigenic peptide is fused to either the first polypeptide or the second polypeptide, and

wherein either the first polypeptide or the second polypeptide, but not both, further comprises a cytoplasmic signaling domain.

2. The MHC-based chimeric receptor of claim 1, which further comprises at least one co-stimulatory domain in the first polypeptide and/or the second polypeptide.

3. The MHC-based chimeric receptor of claim 2, wherein the at least one co-stimulatory domain is a co-stimulatory domain from 4-1BB (CD137), a co-stimulatory domain from CD28, or a combination thereof.

4. The MHC-based chimeric receptor of any one of claims 1-3, which further comprises a hinge domain located between the antigenic peptide and the extracellular domain of the MHC Class II molecule fused to the antigenic peptide.

5. The MHC-based chimeric receptor of any one of claims 1-4, which comprises a cytoplasmic signaling domain of CD3ζ.

6. The MHC-based chimeric receptor of any one of claims 1-3, wherein the antigenic peptide is from myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), proteolipid protein (PLP), insulin or glutamate decarboxylase.

7. The MHC-based chimeric receptor of claim 1, wherein the first class II MHC, the second class II MHC, or both are human MHC II molecules.

8. The MHC-based chimeric receptor of claim 1, wherein the first MHC class II is

human leukocyte antigen (HLA) DR alpha chain HLA-DRA*1010.

9. The MHC-based chimeric receptor of claim 1, wherein the second MHC class II is human leukocyte antigen (HLA) DR beta chain HLA-DRB1*1501.