

treating chronic viral infection (Pharmacologic administration of interleukin-2. Chavez, A.R., et al., Ann N Y Acad Sci, 2009. 1182: 14-27).

As important factors affecting the function of T cells, CTLA4 and CTLA4 mAbs can produce specific therapeutic effect on diseases by interfering with the immune microenvironment in the body. They have high efficacy and remedy the deficiency of traditional medication, opening a novel pathway of gene therapy. CTLA4 and CTLA4 mAbs are being tested in experiments and various stages of clinical trials. For example, in autoimmune diseases, they effectively inhibited airway hyperresponsiveness in an animal model of asthma, prevented the development of rheumatic diseases, mediated immune tolerance to an allograft in the body, and the like. On the other hand, although biological gene therapy has not shown any adverse effect in short term clinical trials, attention should be paid to the potential effect after long term application. For example, excessive blockade of CTLA4-B7 signaling by CTLA4 mAbs may result in the development of autoimmune diseases. As antibodies can specifically bind to their ligands and induce the lysis of target cells or block the progress of pathology, development and utilization of drugs based on antibodies, especially humanized antibodies have important significance in the clinical treatment of malignant tumors and other immune diseases in humans.

At present, there is yet a need to develop novel antibodies blocking the binding of CTLA4 to B7, and their humanized antibodies.

Summary

In one aspect, there is provided an antibody or antigen binding fragment thereof that binds to human cytotoxic T lymphocyte associated antigen 4 ("CTLA4"), comprising a heavy chain variable region and a light chain variable region, wherein

(a) the heavy chain variable region comprises:

- an HCDR1 comprising the amino acid sequence of SEQ ID NO: 27,
- an HCDR2 comprising the amino acid sequence of SEQ ID NO: 28, and
- an HCDR3 comprising the amino acid sequence of SEQ ID NO: 29; and

(b) the light chain variable region comprises:

- an LCDR1 comprising the amino acid sequence of SEQ ID NO: 30,
- an LCDR2 comprising the amino acid sequence of SEQ ID NO: 31, and
- an LCDR3 comprising an amino acid sequence selected from SEQ ID NO: 32, SEQ ID NO: 33, and SEQ ID NO: 34.

In another aspect, there is provided an antibody that binds to human CTLA4 comprising a heavy chain variable region of SEQ ID NO:14, wherein the methionine at position 18 of SEQ ID NO: 14 is substituted with leucine, and a light chain variable region of SEQ ID NO:16.

In another aspect, there is provided an isolated nucleic acid molecule encoding:

- (i) a heavy chain variable region of an antibody or antigen binding fragment thereof, wherein
 - (a) the isolated nucleic acid molecule encodes a heavy chain variable region comprising the amino acid sequence set forth in any of SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 18, or any of SEQ ID NO: 6, 10, 14, or 18 wherein the methionine at position 18 is substituted with leucine, valine, isoleucine or alanine; or
 - (b) the isolated nucleic acid molecule has the nucleotide sequence as set forth in SEQ ID NO: 5, SEQ ID NO: 9, SEQ ID NO: 13, or SEQ ID NO: 17, and
- (ii) a light chain variable region of an antibody or antigen binding fragment thereof, wherein
 - (a) the light chain variable region of the antibody has the amino acid sequence as set forth in SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO: 16, SEQ ID NO: 20, SEQ ID NO: 22 or SEQ ID NO: 24; and
 - (b) the isolated nucleic acid molecule has the nucleotide sequence as set forth in SEQ ID NO: 7, SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 21 or SEQ ID NO: 23.

In another aspect, there is provided a vector comprising the isolated nucleic acid molecule as defined herein.

In another aspect, there is provided a host cell comprising the vector as defined herein.

In another aspect, there is provided a method of preparing the antibody or antigen binding fragment thereof as defined herein, comprising the steps of culturing the host cell of as defined herein under suitable conditions and recovering the antibody or antigen binding fragment thereof from the cell culture.

In another aspect, there is provided a pharmaceutical composition comprising the antibody or antigen binding fragment thereof as defined herein, and a pharmaceutically acceptable carrier and/or excipient.

In another aspect, there is provided use of the antibody or antigen binding fragment as defined herein in the preparation of a medicament for the treatment of a tumor.

In another aspect, there is provided an in vitro method comprising the step of administering to cells an effective amount of the antibody or antigen binding fragment thereof as defined herein, wherein the method is selected from the following:

- (a) a method of detecting the level of CTLA4 in a sample,
- (b) a method of blocking the binding of CTLA4 to B7,
- (c) a method of regulating the activity of CTLA4 or the level of CTLA4,
- (d) a method of relieving the immunosuppression on the body by CTLA4,
- (e) a method of activating T lymphocytes, or
- (f) a method of increasing the expression of IL-2 in T lymphocytes

In another aspect, there is provided the antibody or antigen binding fragment as defined herein for use in therapy.

Description of the invention

After intensive studies and creative works by the inventors, recombinant CTLA4 was expressed using a mammal cell expression system, and used as the antigen to immune mice. Hybridoma cells were obtained by fusing the mouse splenic cells with myeloma cells. After screening a great number of samples by the inventors, a hybridoma cell line capable of secreting and producing a specific monoclonal antibody which specifically binds CTLA4 and can block the binding of CTLA4 to B7 very effectively, was obtained. Furthermore, humanized antibodies were generated. Thus, the following inventions are provided.

One aspect of the present invention relates to a monoclonal antibody or antigen binding fragment thereof, wherein the monoclonal antibody comprises the complementary determining regions (CDR's) selected from the following:

HCDR1 comprising the amino acid sequence of SEQ ID NO: 27,
HCDR2 comprising the amino acid sequence of SEQ ID NO: 28, and

CLAIMS

1. An antibody or antigen binding fragment thereof that binds to human cytotoxic T lymphocyte associated antigen 4 ("CTLA4"), comprising a heavy chain variable region and a light chain variable region, wherein
 - (a) the heavy chain variable region comprises:
 - an HCDR1 comprising the amino acid sequence of SEQ ID NO: 27,
 - an HCDR2 comprising the amino acid sequence of SEQ ID NO: 28, and
 - an HCDR3 comprising the amino acid sequence of SEQ ID NO: 29; and
 - (b) the light chain variable region comprises:
 - an LCDR1 comprising the amino acid sequence of SEQ ID NO: 30,
 - an LCDR2 comprising the amino acid sequence of SEQ ID NO: 31, and
 - an LCDR3 comprising an amino acid sequence selected from SEQ ID NO: 32, SEQ ID NO: 33, and SEQ ID NO: 34.
2. The antibody or antigen binding fragment according to claim 1, selected from the group consisting of:
 - (a) an antibody or antigen binding fragment thereof comprising: a heavy chain variable region comprising an HCDR1 comprising the amino acid sequence of SEQ ID NO:27, an HCDR2 comprising the amino acid sequence of SEQ ID NO:28, and an HCDR3 comprising the amino acid sequence of SEQ ID NO:29 and a light chain variable region comprising an LCDR1 comprising the amino acid sequence of SEQ ID NO:30, an LCDR2 comprising the amino acid sequence of SEQ ID NO:31, and an LCDR3 comprising the amino acid sequence of SEQ ID NO:32;
 - (b) an antibody or antigen binding fragment thereof comprising: a heavy chain variable region comprising an HCDR1 comprising the amino acid sequence of SEQ ID NO:27, an HCDR2 comprising the amino acid sequence of SEQ ID NO:28, and an HCDR3 comprising the amino acid sequence of SEQ ID NO: 29 and a light chain variable region comprising an LCDR1 comprising the amino acid sequence of SEQ ID NO:30, an LCDR2 comprising the amino acid sequence of SEQ ID NO:31, and an LCDR3 comprising the amino acid sequence of SEQ ID NO:33; and

(c) an antibody or antigen binding fragment thereof comprising: a heavy chain variable region comprising an HCDR1 comprising the amino acid sequence of SEQ ID NO:27, an HCDR2 comprising the amino acid sequence of SEQ ID NO:28, and an HCDR3 comprising the amino acid sequence of SEQ ID NO:29 and a light chain variable region comprising an LCDR1 comprising the amino acid sequence of SEQ ID NO:30, an LCDR2 comprising the amino acid sequence of SEQ ID NO:31, and an LCDR3 comprising the amino acid sequence of SEQ ID NO:34.

3. The antibody or antigen binding fragment of claim 2, which is an antibody comprising a heavy chain variable region comprising an HCDR1 comprising the amino acid sequence of SEQ ID NO:27, an HCDR2 comprising the amino acid sequence of SEQ ID NO:28, and an HCDR3 comprising the amino acid sequence of SEQ ID NO:29 and a light chain variable region comprising an LCDR1 comprising the amino acid sequence of SEQ ID NO:30, an LCDR2 comprising the amino acid sequence of SEQ ID NO:31, and an LCDR3 comprising the amino acid sequence of SEQ ID NO:32.

4. The antibody or antigen binding fragment according to any one of claims 1 to 3, wherein the amino acid sequence of the heavy chain variable region (VH) is selected from SEQ ID NO: 14, SEQ ID NO: 18, SEQ ID NO: 6, SEQ ID NO: 10 and any of SEQ ID NOs: 14, 6, and 10 wherein the methionine at position 18 is substituted with an amino acid selected from leucine, valine, isoleucine or alanine,
and
the amino acid sequence of the light chain variable region (VL) is selected from SEQ ID NO: 16, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 8, and SEQ ID NO: 12.

5. The antibody or antigen binding fragment according to any one of

claims 1 to 4, selected from the group consisting of:

- (a) an antibody or antigen binding fragment thereof comprising a heavy chain variable region of SEQ ID NO: 6 and a light chain variable region of SEQ ID NO: 8;
- (b) an antibody or antigen binding fragment thereof comprising a heavy chain variable region of SEQ ID NO: 10 and a light chain variable region of SEQ ID NO: 12;
- (c) an antibody or antigen binding fragment thereof comprising a heavy chain variable region of SEQ ID NO: 14 and a light chain variable region of SEQ ID NO: 16;
- (d) an antibody or antigen binding fragment thereof comprising a heavy chain variable region of SEQ ID NO: 18 and a light chain variable region of SEQ ID NO: 20;
- (e) an antibody or antigen binding fragment thereof comprising a heavy chain variable region of SEQ ID NO: 14 and a light chain variable region of SEQ ID NO: 22; and
- (f) an antibody or antigen binding fragment thereof comprising a heavy chain variable region of SEQ ID NO: 14 and a light chain variable region of SEQ ID NO: 24
- g) an antibody or antigen binding fragment thereof comprising a heavy chain variable region of SEQ ID NO: 6 wherein the methionine at position 18 is substituted with leucine, valine, isoleucine or alanine, and a light chain variable region of SEQ ID NO:8;
- (h) an antibody or antigen binding fragment thereof comprising a heavy chain variable region of SEQ ID NO: 10 wherein the methionine at position 18 is substituted with leucine, valine, isoleucine or alanine, and a light chain variable region of SEQ ID NO:12;
- (i) an antibody or antigen binding fragment thereof comprising a heavy chain variable region of SEQ ID NO: 14 wherein the methionine at position 18 is substituted with leucine, valine, isoleucine or alanine, and a light chain variable region of SEQ ID NO:16;
- (j) an antibody or antigen binding fragment thereof comprising a heavy chain variable region of SEQ ID NO: 14 wherein the methionine at position 18 is substituted with leucine, valine, isoleucine or alanine, and a light chain variable region of SEQ ID NO:22; and
- (k) an antibody or antigen binding fragment thereof comprising a heavy chain variable region of SEQ ID NO: 14 wherein the methionine at position 18 is substituted with leucine, valine, isoleucine or alanine, and a light chain variable region of SEQ ID NO:24.

6. The antibody or antigen binding fragment according to claim 5, wherein the methionine at position 18 of SEQ ID NO: 6, SEQ ID NO: 10, or SEQ ID NO: 14 is substituted with leucine.
7. An antibody that binds to human CTLA4 comprising a heavy chain variable region of SEQ ID NO:14, wherein the methionine at position 18 of SEQ ID NO: 14 is substituted with leucine, and a light chain variable region of SEQ ID NO:16.
8. The antibody or antigen binding fragment according to any one of claims 1 to 7, which is a humanized antibody or antigen binding fragment thereof.
9. The antibody or antigen binding fragment according to any one of claims 1 to 8, wherein the antibody or antigen binding fragment thereof binds to human CTLA4 with a K_D less than 10^{-5} M, as determined by surface plasmon resonance.
10. The antibody or antigen binding fragment according to any one of claims 1-9, wherein the antibody or antigen fragment thereof:
 - (a) blocks the binding of CTLA4 to B7;
 - (b) regulates the activity of CTLA4;
 - (c) relieves the immunosuppression on the body by CTLA4;
 - (d) activates T lymphocytes, and/or
 - (e) increases the expression of IL-2 in T lymphocytes.
11. An isolated nucleic acid molecule encoding:
 - (i) a heavy chain variable region of an antibody or antigen binding fragment thereof, wherein
 - (a) the isolated nucleic acid molecule encodes a heavy chain variable region comprising the amino acid sequence set forth in any of SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 18, or any of SEQ ID NO: 6, 10, or 14, wherein the

methionine at position 18 is substituted with leucine, valine, isoleucine or alanine; or

(b) the isolated nucleic acid molecule has the nucleotide sequence as set forth in SEQ ID NO: 5, SEQ ID NO: 9, SEQ ID NO: 13, or SEQ ID NO: 17, and
(ii)

a light chain variable region of an antibody or antigen binding fragment thereof, wherein

(a) the isolated nucleic acid molecule encodes a light chain variable region comprising the amino acid sequence as set forth in any of SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO: 16, SEQ ID NO: 20, SEQ ID NO: 22 or SEQ ID NO: 24; or

(b) the isolated nucleic acid molecule has the nucleotide sequence as set forth in SEQ ID NO: 7, SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 21 or SEQ ID NO: 23.

12. The isolated nucleic acid molecule of claim 11, encoding (i) the heavy chain variable region of an antibody comprising the amino acid sequence of SEQ ID NO: 14, wherein the methionine at position 18 of SEQ ID NO: 14 is substituted with leucine, and (ii) the light chain variable region of an antibody comprising the amino acid sequence of SEQ ID NO: 16.

13. A vector comprising the isolated nucleic acid molecule of claim 11 or claim 12.

14. A host cell comprising the vector of claim 13.
15. A method of preparing the antibody or antigen binding fragment thereof according to any one of claims 1 to 10, comprising the steps of culturing the host cell of claim 14 under suitable conditions and recovering the antibody or antigen binding fragment thereof from the cell culture.
16. A pharmaceutical composition comprising the antibody or antigen binding fragment thereof according to any one of claims 1 to 10, and a pharmaceutically acceptable carrier and/or excipient.
17. Use of the antibody or antigen binding fragment according to any one of claims 1 to 10 in the preparation of a medicament for the treatment of a tumor.

18. An in vitro method comprising the step of administering to cells an effective amount of the antibody or antigen binding fragment thereof according to any one of claims 1 to 10, wherein the method is selected from the following:

- (a) a method of detecting the level of CTLA4 in a sample,
- (b) a method of blocking the binding of CTLA4 to B7,
- (c) a method of regulating the activity of CTLA4 or the level of CTLA4,
- (d) a method of relieving the immunosuppression on the body by CTLA4,
- (e) a method of activating T lymphocytes, or
- (f) a method of increasing the expression of IL-2 in T lymphocytes.

19. The antibody or antigen binding fragment according to any one of claims 1 to 10 for use in therapy.