

[Citation List]

[Non Patent Literature]

[0003]

[NPL 1] N Engl J Med 2007; 357:1731-1743

[NPL 2] J Biomed Sci. 2012; 19(1): 88

[NPL 3] N Engl J Med 2003; 348:2517-2524

[NPL 4] Gut 2003; 52:960-965

[NPL 5] Dig Dis Sci 2004; 49:1479-1484

[NPL 6] Gastroenterology 2011; 141:610-620

[NPL 7] Gut 2005; 54:1217-1223

[NPL 8] Gastroenterology 2014; 146:1649-58

[NPL 9] Nutrients 2013 Oct 5(10): 3975-3992

[NPL 10] J Clin Invest. 2007; 117(1):41-49

[NPL 11] Am J Clin Nutr 2007; 85: 160-6

[Summary]

In one aspect, the present disclosure provides a multispecific antigen-binding molecule that binds to a complex formed by HLA-DQ2.5 and a gluten peptide and which comprises

a first antigen-binding moiety comprising first and second antibody variable regions and a second antigen-binding moiety comprising third and fourth antibody variable regions, wherein the multispecific antigen-binding molecule comprises any one of (1) to (14) below:

- (1) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 88; a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 90; a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 95; and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 100;
- (2) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 88; a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 90; a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 92; and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 98;
- (3) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 88; and a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 90; a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 92; and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 99;
- (4) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 88; a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 90; a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 93; and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 99;

- [illegible]

- (13) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 89; a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 91; a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 96; and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 100; and
- (14) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 89; a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 91; a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 97; and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 99.

In another aspect, the present disclosure provides a multispecific antigen-binding molecule that binds to a complex formed by HLA-DQ2.5 and a gluten peptide, comprising a combination of four polypeptide chains selected from the group consisting of (1) to (14) below:

- (1) a first heavy chain comprising amino acid sequence of SEQ ID NO: 42 and a first light chain comprising amino acid sequence of SEQ ID NO: 43, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 63 and a second light chain comprising amino acid sequence of SEQ ID NO: 61;
- (2) a first heavy chain comprising amino acid sequence of SEQ ID NO: 42 and a first light chain comprising amino acid sequence of SEQ ID NO: 43, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 54 and a second light chain comprising amino acid sequence of SEQ ID NO: 55;
- (3) a first heavy chain comprising amino acid sequence of SEQ ID NO: 42 and a first light chain comprising amino acid sequence of SEQ ID NO: 43, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 54 and a second light chain comprising amino acid sequence of SEQ ID NO: 56;
- (4) a first heavy chain comprising amino acid sequence of SEQ ID NO: 42 and a first light chain comprising amino acid sequence of SEQ ID NO: 43, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 58 and a second light chain comprising amino acid sequence of SEQ ID NO: 56;
- (5) a first heavy chain comprising amino acid sequence of SEQ ID NO: 42 and a first light chain comprising amino acid sequence of SEQ ID NO: 43, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 60 and a second light chain comprising amino acid sequence of SEQ ID NO: 61;
- (6) a first heavy chain comprising amino acid sequence of SEQ ID NO: 45 and a first light chain comprising amino acid sequence of SEQ ID NO: 46, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 54 and a second light chain comprising amino acid sequence of SEQ ID NO: 55;

- (7) a first heavy chain comprising amino acid sequence of SEQ ID NO: 45 and a first light chain comprising amino acid sequence of SEQ ID NO: 46, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 65 and a second light chain comprising amino acid sequence of SEQ ID NO: 61;
- (8) a first heavy chain comprising amino acid sequence of SEQ ID NO: 45 and a first light chain comprising amino acid sequence of SEQ ID NO: 46, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 54 and a second light chain comprising amino acid sequence of SEQ ID NO: 56;
- (9) a first heavy chain comprising amino acid sequence of SEQ ID NO: 45 and a first light chain comprising amino acid sequence of SEQ ID NO: 46, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 58 and a second light chain comprising amino acid sequence of SEQ ID NO: 56;
- (10) a first heavy chain comprising amino acid sequence of SEQ ID NO: 45 and a first light chain comprising amino acid sequence of SEQ ID NO: 46, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 67 and a second light chain comprising amino acid sequence of SEQ ID NO: 56;
- (11) a first heavy chain comprising amino acid sequence of SEQ ID NO: 42 and a first light chain comprising amino acid sequence of SEQ ID NO: 43, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 65 and a second light chain comprising amino acid sequence of SEQ ID NO: 61;
- (12) a first heavy chain comprising amino acid sequence of SEQ ID NO: 42 and a first light chain comprising amino acid sequence of SEQ ID NO: 43, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 67 and a second light chain comprising amino acid sequence of SEQ ID NO: 56;
- (13) a first heavy chain comprising amino acid sequence of SEQ ID NO: 45 and a first light chain comprising amino acid sequence of SEQ ID NO: 46, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 63 and a second light chain comprising amino acid sequence of SEQ ID NO: 61; and
- (14) a first heavy chain comprising amino acid sequence of SEQ ID NO: 45 and a first light chain comprising amino acid sequence of SEQ ID NO: 46, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 60 and a second light chain comprising amino acid sequence of SEQ ID NO: 61.

In another aspect, the present disclosure provides a nucleic acid encoding the multispecific antigen-binding molecule of the disclosure.

In another aspect, the present disclosure provides a vector comprising the nucleic acid of the disclosure.

In another aspect, the present disclosure provides a cell comprising the nucleic acid of the disclosure.

In another aspect, the present disclosure provides a method of producing a multispecific antigen-binding molecule comprising culturing the cell of the disclosure so that the multispecific antigen-binding molecule is produced.

In another aspect, the present disclosure provides a pharmaceutical composition comprising the multispecific antigen-binding molecule of the disclosure, and a pharmaceutically acceptable carrier.

In another aspect, the present disclosure provides a use of the multispecific antigen-binding molecule of the disclosure in the manufacture of a medicament for treating and/or preventing celiac disease.

[Technical Problem]

[0004]

Under the above-mentioned circumstances with the need for adjunctive therapy, the present invention provides anti-HLA-DQ2.5 antigen-binding molecules.

[Solution to Problem]

[0005]

The antigen-binding molecules of the present invention have been altered and can bind to two or more complexes formed by HLA-DQ2.5 and a gluten peptide.

[0006]

More specifically, the present invention provides the following.

[1] A multispecific antigen-binding molecule comprising:

(i) a first antigen-binding moiety that has binding activity to HLA-DQ2.5 in the form of a complex with a gluten peptide; and

(ii) a second antigen-binding moiety that has binding activity to HLA-DQ2.5 in the form of a complex with a gluten peptide;

wherein the antigen-binding molecule binds to two or more complexes of HLA-DQ2.5 and gluten peptides,

wherein at least one of the gluten peptides in the complexes bound by the first antigen-binding moiety is different from at least one of the gluten peptides in the complexes bound by the second antigen-binding moiety; and

wherein the antigen-binding molecule has substantially no binding activity to either or both of a HLA-DQ2.5 positive PBMC B cell and a Ba/F3 cell that expresses HLA-DQ2.5,

wherein the antigen-binding molecule is humanized, and

wherein one or more amino acids in a heavy chain and/or a light chain of the first

of SEQ ID NO: 94; and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 100;

(12) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 89; a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 91; a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 95; and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 100;

(13) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 89; a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 91; a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 96; and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 100;

(14) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 89; a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 91; a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 97; and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 99;

(15) a first amino acid sequence that has at least 70%, 75%, 80%, 85%, 90%, or 95% sequence identity to the first antibody variable region recited in any one of (1) to (14); a second amino acid sequence that has at least 70%, 75%, 80%, 85%, 90%, or 95% sequence identity to the second antibody variable region recited in any one of (1) to (14); a third amino acid sequence that has at least 70%, 75%, 80%, 85%, 90%, or 95% sequence identity to the third antibody variable region recited in any one of (1) to (14); and a fourth amino acid sequence that has at least 70%, 75%, 80%, 85%, 90%, or 95% sequence identity to the fourth antibody variable region recited in any one of (1) to (14).

[27] A multispecific antigen-binding molecule comprising a combination of two polypeptide chains selected from the group consisting of (A1) to (A3) below:

(A1) a first heavy chain comprising amino acid sequence of SEQ ID NO: 42 and a first light chain comprising amino acid sequence of SEQ ID NO: 43;

(A2) a first heavy chain comprising amino acid sequence of SEQ ID NO: 45 and a first light chain comprising amino acid sequence of SEQ ID NO: 46; and

(A3) a first amino acid sequence that has at least 70%, 75%, 80%, 85%, 90%, or 95% sequence identity to the first heavy chain sequence recited in (A1) or (A2), and a second amino acid sequence that has at least 70%, 75%, 80%, 85%, 90%, or 95% sequence identity to the first light chain sequence recited in (A1) or (A2).

[27-2] A multispecific antigen-binding molecule comprising a combination of two polypeptide chains selected from the group consisting of (A1) to (A3) below:

(A1) a first heavy chain comprising amino acid sequence of SEQ ID NO: 41 and a first

sequence of SEQ ID NO: 99;

(10) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 89; a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 91; a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 93; and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 99;

(11) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 89; a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 91; a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 94; and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 100;

(12) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 89; a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 91; a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 95; and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 100;

(13) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 89; a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 91; a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 96; and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 100;

(14) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 89; a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 91; a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 97; and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 99;

(15) a first amino acid sequence that has at least 70%, 75%, 80%, 85%, 90%, or 95% sequence identity to the first antibody variable region recited in any one of (1) to (14); a second amino acid sequence that has at least 70%, 75%, 80%, 85%, 90%, or 95% sequence identity to the second antibody variable region recited in any one of (1) to (14); a third amino acid sequence that has at least 70%, 75%, 80%, 85%, 90%, or 95% sequence identity to the third antibody variable region recited in any one of (1) to (14); and a fourth amino acid sequence that has at least 70%, 75%, 80%, 85%, 90%, or 95% sequence identity to the fourth antibody variable region recited in any one of (1) to (14). In some embodiments, the present disclosure provides a multispecific antigen-binding molecule comprising a combination of two polypeptide chains selected from the group consisting of (A1) to (A3) below:

(A1) a first heavy chain comprising amino acid sequence of SEQ ID NO: 42 and a first

[CLAIMS]

[Claim 1]

A multispecific antigen-binding molecule that binds to a complex formed by HLA-DQ2.5 and a gluten peptide and which comprises

a first antigen-binding moiety comprising first and second antibody variable regions and a second antigen-binding moiety comprising third and fourth antibody variable regions, wherein the multispecific antigen-binding molecule comprises any one of (1) to (14) below:

- (1) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 88; a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 90; a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 95; and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 100;
- (2) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 88; a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 90; a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 92; and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 98;
- (3) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 88; and a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 90; a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 92; and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 99;
- (4) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 88; a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 90; a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 93; and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 99;
- (5) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 88; a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 90; a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 94; and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 100;
- (6) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 88; a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 90; a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 96; a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 100;
- (7) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 88; a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 90; a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 97; and a fourth

- [illegible]

[Claim 2] A multispecific antigen-binding molecule that binds to a complex formed by HLA-DQ2.5 and a gluten peptide, comprising a combination of four polypeptide chains selected from the group consisting of (1) to (14) below:

- (1) a first heavy chain comprising amino acid sequence of SEQ ID NO: 42 and a first light chain comprising amino acid sequence of SEQ ID NO: 43, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 63 and a second light chain comprising amino acid sequence of SEQ ID NO: 61;
- (2) a first heavy chain comprising amino acid sequence of SEQ ID NO: 42 and a first light chain comprising amino acid sequence of SEQ ID NO: 43, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 54 and a second light chain comprising amino acid sequence of SEQ ID NO: 55;
- (3) a first heavy chain comprising amino acid sequence of SEQ ID NO: 42 and a first light chain comprising amino acid sequence of SEQ ID NO: 43, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 54 and a second light chain comprising amino acid sequence of SEQ ID NO: 56;
- (4) a first heavy chain comprising amino acid sequence of SEQ ID NO: 42 and a first light chain comprising amino acid sequence of SEQ ID NO: 43, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 58 and a second light chain comprising amino acid sequence of SEQ ID NO: 56;
- (5) a first heavy chain comprising amino acid sequence of SEQ ID NO: 42 and a first light chain comprising amino acid sequence of SEQ ID NO: 43, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 60 and a second light chain comprising amino acid sequence of SEQ ID NO: 61;
- (6) a first heavy chain comprising amino acid sequence of SEQ ID NO: 45 and a first light chain comprising amino acid sequence of SEQ ID NO: 46, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 54 and a second light chain comprising amino acid sequence of SEQ ID NO: 55;
- (7) a first heavy chain comprising amino acid sequence of SEQ ID NO: 45 and a first light chain comprising amino acid sequence of SEQ ID NO: 46, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 65 and a second light chain comprising amino acid sequence of SEQ ID NO: 61;
- (8) a first heavy chain comprising amino acid sequence of SEQ ID NO: 45 and a first light chain comprising amino acid sequence of SEQ ID NO: 46, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 54 and a second light chain comprising amino acid sequence of SEQ ID NO: 56;
- (9) a first heavy chain comprising amino acid sequence of SEQ ID NO: 45 and a first light chain comprising amino acid sequence of SEQ ID NO: 46, and a second heavy chain comprising amino acid sequence of SEQ ID

- NO: 58 and a second light chain comprising amino acid sequence of SEQ ID NO: 56;
- (10) a first heavy chain comprising amino acid sequence of SEQ ID NO: 45 and a first light chain comprising amino acid sequence of SEQ ID NO: 46, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 67 and a second light chain comprising amino acid sequence of SEQ ID NO: 56;
 - (11) a first heavy chain comprising amino acid sequence of SEQ ID NO: 42 and a first light chain comprising amino acid sequence of SEQ ID NO: 43, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 65 and a second light chain comprising amino acid sequence of SEQ ID NO: 61;
 - (12) a first heavy chain comprising amino acid sequence of SEQ ID NO: 42 and a first light chain comprising amino acid sequence of SEQ ID NO: 43, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 67 and a second light chain comprising amino acid sequence of SEQ ID NO: 56;
 - (13) a first heavy chain comprising amino acid sequence of SEQ ID NO: 45 and a first light chain comprising amino acid sequence of SEQ ID NO: 46, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 63 and a second light chain comprising amino acid sequence of SEQ ID NO: 61; and
 - (14) a first heavy chain comprising amino acid sequence of SEQ ID NO: 45 and a first light chain comprising amino acid sequence of SEQ ID NO: 46, and a second heavy chain comprising amino acid sequence of SEQ ID NO: 60 and a second light chain comprising amino acid sequence of SEQ ID NO: 61.

[Claim 3] The multispecific antigen-binding molecule of claim 1 or 2, which is a bispecific antibody.

[Claim 4]

A nucleic acid encoding the multispecific antigen-binding molecule of any one of claims 1 to 3.

[Claim 5]

A vector comprising the nucleic acid of claim 4.

[Claim 6]

A cell comprising the nucleic acid of claim 4 or the vector of claim 5.

[Claim 7]

A method of producing a multispecific antigen-binding molecule comprising culturing the cell of claim 6 so that the multispecific antigen-binding molecule is produced.

[Claim 8]

The method of claim 7, further comprising recovering the multispecific antigen-binding molecule from the culture of the cell.

[Claim 9]

A pharmaceutical composition comprising the multispecific antigen-binding molecule of any one of claims 1 to 3, and a pharmaceutically acceptable carrier.

[Claim 10]

The composition of claim 9, which is a pharmaceutical composition for use in therapy.
[Claim 11]

Use of the multispecific antigen-binding molecule of any one of claims 1 to 3 in the manufacture of a medicament for treating and/or preventing celiac disease.