have very low affinity to muramyl peptides. Inhibition assays using mAb2-4 showed that 50% inhibition of mAb2-4 binding to peptidoglycan by N-acetylmuramyl-L-alanyl-D-isoglutamine occurred only at concentrations higher than 1 mg/ml. Additionally, structural analysis of antigenic determinant showed that the mAb2-4 antibody recognizes the N-acetylmuramic acid linked to the dipeptide. As many bacterial species do not have the N-acetyl group in the muramic acid residue, mAb2-4 appears to be an antibody that has narrow specificity in recognizing bacterial species.

[009] Another commonly used mouse monoclonal antibody for peptidoglycan is mAb2E9, which was developed by immunizing mice with partially purified peptidoglycan-polysaccharide complexes isolated from the feces of a healthy human. The affinity of this antibody to N-acetylmuramyl-L-alanyl-D-isoglutamine was found to be even lower than mAb2-4, and the antigenic determinant has not been defined. mAb2E9 has been used in immunostaining of tissues but never in ELISA.

[0010] Other monoclonal antibodies developed by immunizing mice with peptidoglycan isolated from *Streptococcus mutans* have also been described. Although these antibodies could recognize peptidoglycan prepared from multiple bacterial species including both Gram-positive and Gram-negative ones, the binding of such antibodies are known to be non-specific as they cannot be inhibited by N-acetylmuramyl-L-alanyl-D-isoglutamine and the antigenic determinant is not known.

[0011] In view of the low affinity, poorly defined antigenic determinant and narrow specificity of the currently available mouse monoclonal antibodies, there is a need to provide an alternative antibody that recognizes muramyl peptide.

SUMMARY

[0012] Disclosed is an isolated antibody or an antigen-binding fragment thereof capable of binding to a muramyl peptide, or a derivative or an analog or a salt thereof, comprising muramic acid and an amino acid selected from the group consisting of alanine, isoglutamine, glutamic acid, and a salt thereof.

[0013] Disclosed is an isolated antibody or an antigen-binding fragment thereof comprising a heavy chain variable domain comprising the amino acid sequence as set forth in SEQ ID NO: 3, or a variant thereof.

[0014] Disclosed is an isolated antibody or an antigen-binding fragment thereof comprising a light chain variable domain comprising the amino acid sequence as set forth in SEQ ID NO: 4, or a variant thereof.

[0015] Disclosed is an isolated antibody or an antigen-binding fragment thereof comprising a heavy chain variable domain comprising the amino acid sequence as set forth in SEQ ID NO: 3, or a variant thereof, and a light chain variable domain comprising the amino acid sequence as set forth in SEQ ID NO: 4, or a variant thereof.

[0015a] In one aspect, there is provided an isolated antibody or an antigen-binding fragment thereof, capable of binding to a muramyl peptide selected from the group consisting of N-acetylmuramyl-L-alanyl-D-isoglutamine, muramyl-L-alanyl-D-isoglutamine, N-acetylmuramyl-L-alanyl-D-glutamate, and muramyl-L-alanyl-D-glutamate; wherein the isolated antibody or an antigen-binding fragment thereof comprises a heavy chain variable domain comprising the amino acid sequence as set forth in SEQ ID NO: 3, and a light chain variable domain comprising the amino acid sequence as set forth in SEQ ID NO: 4.

[0016] Disclosed is an isolated nucleic acid molecule comprising SEQ ID NO: 1 or SEQ ID NO: 2.

[0017] Disclosed is a vector comprising the isolated nucleic acid molecule as described herein.

[0018] Disclosed is a host cell comprising the nucleic acid molecule as described herein or the vector as described herein.

[0019] Disclosed is an isolated cell line that produces the antibody or an antigen-binding fragment thereof as described herein.

[0020] Disclosed is a method of producing an antibody or an antigen-binding fragment thereof as described herein, the method comprising the steps of culturing the host cell as described herein or the isolated cell line as described herein under suitable conditions and recovering the antibody or antigen-binding fragment thereof.

[0021] Disclosed is a method of producing an antibody or an antigen-binding fragment thereof as described herein, comprising the steps of inoculating a muramyl peptide, or a derivative or an analog or a salt thereof, as described herein into a non-human animal, and isolating antibodies or antigen-binding fragments produced therefrom.

[0022] Disclosed is a composition comprising an antibody or an antigen-binding fragment thereof as described herein, and a pharmaceutically acceptable carrier.

[0023] Disclosed is a vaccine comprising a muramyl peptide, or a derivative or an analog or a salt thereof, as described herein.

[0024] Disclosed is an immunoconjugate comprising an antibody or an antigen-binding fragment thereof as described herein, covalently attached to: a

cytotoxic agent selected from the group consisting of an antibacterial agent, a chemotherapeutic agent, a drug moiety, an antibiotic, a radioisotope, a nucleolytic enzyme, a lysozyme, a proteinase and a lipase; or a capture label; or a detection label selected from the group consisting of fluorescein, rhodamine, dansyl, Lissamine, cyanine, phycoerythrin, Texas Red, and a radionuclide detection label.

[0025] Disclosed is a method of prophylactically or therapeutically treating an autoimmune or inflammatory disease selected from the group consisting of sepsis, septic shock, Crohn's disease, rheumatoid arthritis, asthma, allergy, atopic disorders, multiple sclerosis, pertussis, gonorrhea, inflammatory bowel disease, and antibiotic-associated disorder, comprising administering to a subject an isolated antibody or an antigen-binding fragment thereof as described herein, a composition as described herein, or an immunoconjugate as described herein.

[0026] Disclosed is a method of prophylactically or therapeutically treating a bacterial infection, comprising administering to a subject an isolated antibody or an antigen-binding fragment thereof as described herein, a composition as described herein, or an immunoconjugate as described herein.

[0027] Disclosed is an isolated antibody or an antigen-binding fragment thereof as described herein, a composition as described herein, or an immunoconjugate as described herein, for use in the prophylactic or therapeutic treatment of an autoimmune or inflammatory disease selected from the group consisting of sepsis, septic shock, Crohn's disease, rheumatoid arthritis, asthma, allergy, atopic disorders, multiple sclerosis, pertussis, gonorrhea, inflammatory bowel disease, and antibiotic-associated disorder.

[0028] Disclosed is an isolated antibody or an antigen-binding fragment thereof as described herein, a composition as described herein, or an immunoconjugate as described herein, for use in the prophylactic or therapeutic treatment of a bacterial infection.

[0029] Disclosed is use of an isolated antibody or an antigen-binding fragment thereof as described herein, a composition as described herein, or an immunoconjugate as described herein, in the manufacture of a medicament for prophylactically or therapeutically treating an autoimmune or inflammatory disease selected from the group consisting of sepsis, septic shock, Crohn's disease,

rheumatoid arthritis, asthma, allergy, atopic disorders, multiple sclerosis, pertussis, gonorrhea, inflammatory bowel disease, and antibiotic-related disorder.

[0030] Disclosed is use of an isolated antibody or an antigen-binding fragment thereof as described herein, a composition as described herein, or an immunoconjugate as described herein, in the manufacture of a medicament for prophylactically or therapeutically treating a bacterial infection.

[0031] Disclosed is a method of detecting the presence or absence of a muramyl peptide, or a derivative or an analog or a salt thereof, as described herein, in a sample, the method comprising contacting the sample with an isolated antibody or an antigen-binding fragment thereof as described herein, a composition as described herein, or an immunoconjugate as described herein, and analyzing the sample to determine the presence or absence of the binding product of the muramyl peptide, or a derivative or an analog or a salt thereof, and the isolated antibody or an antigen-binding fragment thereof as described herein.

[0032] Disclosed is an assay for detecting a muramyl peptide, or a derivative or an analog or a salt thereof, as described herein, comprising exposing a sample to an antibody or an antigen-binding fragment thereof as described herein, a composition as described herein, or an immunoconjugate as described herein, and determining the extent of binding of the antibody, or an antigen-binding fragment thereof, the composition or the immunoconjugate to the sample.

[0033] Disclosed is a kit for use in a method as described herein, or in an assay as described herein, comprising an isolated antibody or an antigen-binding fragment thereof as described herein, a composition as described herein, or an immunoconjugate as described herein, and one or more reagents for determining the presence or absence of the binding product of a muramyl peptide, or a derivative or an analog or a salt thereof, and the isolated antibody or an antigen-binding fragment thereof as described herein.

[0034] Disclosed is an antibacterial agent comprising an isolated antibody or an antigen-binding fragment thereof as described herein, a composition as described herein, or an immunoconjugate as described herein.

[0035] In another aspect, there is provided a hybridoma deposited as Wy-Hyb-2E7 under Accession Number No. PTA-13256 in the American Type Culture Collection (ATCC), Manassas, VA, USA.

[0036] Disclosed is an antibody comprising the variable heavy and variable light domain sequence of the antibody produced by the hybridoma deposited as Wy-Hyb-2E7 under Accession Number No. PTA-13256 in the American Type Culture Collection (ATCC), Manassas, VA, USA.

BRIEF DESCRIPTION OF THE DRAWINGS

[0037] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0038] **Fig. 1** shows the determination of the Kd value of one example of an antibody as described herein, which is the monoclonal antibody 2E7. To determine the Kd of 2E7 to N-acetylmuramyl-L-alanyl-D-glutamine, the bottom of wells of a 96-well plate was coated with MDP-OVA.. 2-fold serial dilutions of 2E7 were added to the wells for incubation. After washes to remove unbound Abs, an anti-mouse-IgG Ab coupled with horseradish peroxidase (HSP) was added. After incubation, unbound secondary Ab was removed by washes and a chromogenic substrate of HSP was added. After reaction, the color intensity in each well was measured using a microtiter plate reader at 492 nm. A binding curve was generated using CurveExpert Basic program (http://www.curveexpert.net) by fitting 2E7 concentrations and OD492 values to the Weibull Model equation y = a – b*exp(-C*x^d) using CurveExpert Basic. r: correlation coefficient; s: standard error. Coefficient data: a=2.8768241, b=2.8055033, c=212.44541, and d=0.8659833. Y_{50}=1.45, X=0.00131 µg/ml. Kd=0.0131/[150 x10^9] = 8.7 pM (IgG MW = 150).

[0039] **Fig. 2** (A) shows a standard curve that was generated by fitting N-acetylmuramyl-L-alanyl-D-isoglutamine concentrations and OD_{492} values to the Shifted Power Fit equation y = a*(x-b)^c using CurveExpert Basic. The standard curve was used to determine the peptidoglycan concentration in a sample. (B) shows a graph showing *Staphylococcus aureus* and *Escherichia coli* growth in liquid LB medium in the presence or absence of amoxicillin. Both bacteria were grown to OD_{600} = 1.5. Each culture was divided into two, and one treated with 40 µg/ml amoxicillin (Amx) and the other untreated. Aliquots were collected at the

Claims

1. An isolated antibody or an antigen-binding fragment thereof, capable of binding to a muramyl peptide selected from the group consisting of N-acetylmuramyl-L-alanyl-D-isoglutamine, muramyl-L-alanyl-D-isoglutamine, N-acetylmuramyl-L-alanyl-D-glutamate, and muramyl-L-alanyl-D-glutamate;

wherein the isolated antibody or an antigen-binding fragment thereof comprises a heavy chain variable domain comprising the amino acid sequence as set forth in SEQ ID NO: 3, and a light chain variable domain comprising the amino acid sequence as set forth in SEQ ID NO: 4.

2. The isolated antibody or an antigen-binding fragment thereof according to claim 1, comprising a heavy chain encoded by the nucleotide sequence of SEQ ID NO: 1.

3. The isolated antibody or an antigen-binding fragment thereof according to claim 1, comprising a light chain encoded by the nucleotide sequence of SEQ ID NO: 2.

4. The isolated antibody or an antigen-binding fragment thereof according to claim 1, comprising a heavy chain encoded by the nucleotide sequence of SEQ ID NO: 1 and a light chain encoded by the nucleotide sequence of SEQ ID NO: 2.

5. The isolated antibody or an antigen-binding fragment thereof according to claim 1, wherein said antibody or antigen-binding fragment binds to said muramyl peptide, or a derivative or an analog or a salt thereof, with a Kd value selected from the group consisting of less than about 1 nM, less than about 900 pM, less than about 800 pM, less than about 700 pM, less than about 600 pM, less than about 500 pM, less than about 400 pM, less than about 300 pM, less than about 200 pM, less than about 100 pM, less than about 90 pM, less than about 80 pM, less than about 70 pM, less than about 60 pM, less than about 50 pM, less than about 40 pM, less than about 30 pM, less than about 20 pM, and less than about 10 pM.

6. The isolated antibody or an antigen-binding fragment thereof according to claim 1, covalently attached to:

a cytotoxic agent selected from the group consisting of an antibacterial agent, a chemotherapeutic agent, a drug moiety, an antibiotic, a radioisotope, a nucleolytic enzyme, a lysozyme, a proteinase and a lipase; or

a capture label; or

a detection label selected from the group consisting of fluorescein, rhodamine, dansyl, Lissamine, cyanine, phycoerythrin, Texas Red, and a radionuclide detection label, in the form of an immunoconjugate.

7. The isolated antibody or an antigen-binding fragment thereof according to claim 1, as an antibacterial agent.

8. A hybridoma deposited as Wy-Hyb-2E7 under Accession Number No. PTA-13256 in the American Type Culture Collection (ATCC), Manassas, VA, USA.

9. The isolated antibody or an antigen-binding fragment thereof according to claim 1, comprising the variable heavy and variable light domain sequence of the antibody produced by the hybridoma deposited as Wy-Hyb-2E7 under Accession Number No. PTA-13256 in the American Type Culture Collection (ATCC), Manassas, VA, USA.