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(54) Title: ANTIBODIES AGAINST FLAGELLIN AND USES THEREOF

(57) Abstract: The present invention provides a novel class of monoclonal antibodies which have a high affinity, broad spectrum neutralizing reactivity to flagellin from various Gram-negative bacteria including, but not limited to, *E. coli*, *Salmonella*, *Serratia*, *Proteus*, *Enterobacter*, *Citrobacter*, *Campylobacter* and *Pseudomonas*. The present invention further provides methods of treating inflammatory bowel disease (IBD) and methods of treating enterobacterial infections using anti-flagellin antibodies in humans, other animals and birds.

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1. An isolated monoclonal antibody that binds to flagellin comprising a heavy chain variable region comprising an amino acid sequence set forth in SEQ ID NO:2 or an amino acid sequence at least 80% identical thereto.
2. An isolated monoclonal antibody that binds to flagellin comprising a light chain variable region comprising an amino acid sequence set forth in SEQ ID NO:4 or an amino acid sequence at least 80% identical thereto.
3. An isolated monoclonal antibody that binds to flagellin comprising a heavy and light chain variable region comprising the amino acid sequences set forth in SEQ ID NOs:2 and 4, respectively, or amino acid sequences at least 80% identical thereto.
4. An isolated monoclonal antibody that binds to flagellin comprising a heavy and light chain variable region comprising the amino acid sequences set forth in SEQ ID NOs:2 and 4, respectively.
5. An isolated monoclonal antibody that binds to flagellin and comprises heavy and light chain variable region CDR1, CDR2 and CDR3, wherein the
 - heavy chain variable region CDR1 comprises SEQ ID NO:6;
 - a heavy chain variable region CDR2 comprises SEQ ID NO: 8;
 - a heavy chain variable region CDR3 comprises SEQ ID NO: 10;
 - a light chain variable region CDR1 comprises SEQ ID NO: 12;
 - a light chain variable region CDR2 comprises SEQ ID NO: 14; and
 - a light chain variable region CDR3 comprises SEQ ID NO: 16.
6. An isolated antibody that binds to an epitope on flagellin recognized by an antibody comprising heavy and light chain variable regions comprising the amino acid sequences set forth in SEQ ID NOs: 2 and 4, respectively.
7. An isolated antibody that cross competes for binding to flagellin with an antibody comprising heavy and light chain variable regions comprising the amino acid sequences set forth in SEQ ID NOs: 2 and 4, respectively.

8. The antibody of claim 6 or 7, wherein the antibody specifically binds to an epitope located between amino acids 30-50 of flagellin from *Salmonella* (SEQ ID NO:39) or *Pseudomonas* (SEQ ID NO:36).
9. The antibody of claim 8, wherein the antibody specifically binds to an epitope located between amino acids 37-43 of flagellin from *Salmonella* (SEQ ID NO:39) or *Pseudomonas* (SEQ ID NO:36).
10. The antibody of any of the preceding claims, wherein the antibody is selected from the group consisting of a human antibody, a humanized antibody, a bispecific antibody and a chimeric antibody.
11. The antibody of any of the preceding claims, wherein the antibody is selected from the group consisting of a Fab, Fab'2, ScFv, SMIP, affibody, avimer, nanobody, and a domain antibody.
12. The antibody of any of the preceding claims, wherein the antibody isotype is selected from the group consisting of an IgG1, an IgG2, an IgG3, an IgG4, an IgM, an IgA1, an IgA2, an IgAsec, an IgD, and an IgE antibody.
13. A hybridoma which produces an antibody of any of claims 1-5.
14. An immunoconjugate comprising the antibody of any of claims 1-5 linked to a therapeutic agent.
15. The immunoconjugate of claim 14, wherein the therapeutic agent is selected from the group consisting of a cytotoxic agent, an immunosuppressive agent, and a chemotherapeutic agent.
16. A bispecific molecule comprising the antibody of any of claims 1-5 linked to an molecule having a binding specificity which is different from said antibody.

17. A composition comprising the antibody of claims 1-5, an immunoconjugate of any of claims 14-15, or the bispecific molecule of claim 16, and a pharmaceutically effective carrier.
18. The composition of claim 17, further comprising a therapeutic agent.
19. The composition of claim 18, wherein the therapeutic agent is a second antibody.
20. The composition of claim 18, wherein the therapeutic agent is an antibiotic.
21. An isolated nucleic acid molecule encoding a variable region of an antibody that binds to flagellin, wherein the nucleic acid comprises the nucleotide sequence set forth in SEQ ID NOs: 20 or 22 or a nucleotide sequence at least 80% identical thereto.
22. An isolated nucleic acid molecule encoding a variable region of an antibody that binds to flagellin, wherein the nucleic acid comprises a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence set forth in SEQ ID NOs: 20 or 22.
23. An isolated nucleic acid molecule encoding a variable region of an antibody that binds to flagellin, wherein the variable region comprises an amino acid sequence set forth in SEQ ID NOs: 20 or 22.
24. An expression vector comprising the nucleic acid molecule of any of claims 21-23.
25. A kit comprising one or more isolated monoclonal antibodies of any of claims 1-5, and optionally comprising instructions for use in treating or diagnosing a disease or infection associated with flagellin.
26. An isolated cell expressing the antibody of any of claims 1-7.
27. A method of producing the antibody of any of claims 1-7, the method comprising (a) providing the cell of claim 26 and (b) isolating the antibody from the cell, thereby producing the antibody of any of claims 1-7.