SUMMARY ~~OF THE INVENTION~~

[0009] This ~~invention~~disclosure is based on the discovery that the monoclonal antibody F-598 can serve as a complementary therapy to the vaccines disclosed herein for the treatment of PNAG-based microbes. Accordingly, this ~~invention~~disclosure is directed to methods for providing continuous immune protection against PNAG based microbes by co-administration of a oligosaccharide β-(1→6)-glucosamine vaccine and F-598 monoclonal antibody. In one ~~aspect~~example, the vaccine is directed to a specific class of tetra-, penta-, and hexa-β-(1→6)-glucosamine–linked–tetanus toxoid vaccines that provide effective immunity to the patient against microbial infections wherein said microbe comprises PNAG structures in its cell walls.

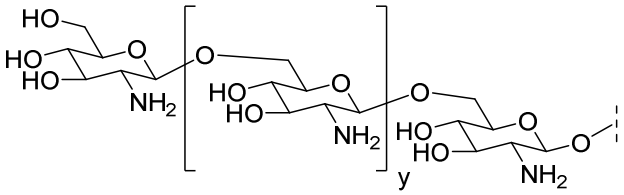
[0010] Surprisingly, while these vaccines generate an endogenous immune response, the oligosaccharide β-(1→6)-glucosamine groups on the vaccine do not appreciably cross-react with the F-598 antibody. This surprising result allows for co-administration of both the vaccine and the antibody. Such co-administration further allows for the clinician to provide continuous complementary immune protection to the patient. In some ~~embodiment~~examples, the complementary immune protection is synergistic.

[0011] Accordingly, in one ~~embodiment~~example, this ~~invention~~disclosure provides for a method for providing continuous immune protection against PNAG microbes by use of a vaccine comprising a β-(1→6)-glucosamine oligosaccharide–linked–tetanus toxoid vaccine that provide effective immunity to a patient against microbial infections wherein said microbe comprises β-(1→6)-glucosamine structures in its cell walls. In one ~~embodiment~~example, the antibodies to the vaccine will bind to β-(1→6)-glucosamine structures. In some ~~embodiment~~examples, said vaccine does not cross-react with a F-598 monoclonal antibody and further wherein said oligosaccharide comprises from 3 to 12 β-(1→6)-glucosamine units. In some ~~embodiment~~examples, the vaccines generate antibodies that are complementary to F-598. That is where the vaccines disclosed herein will selectively bind to β-(1→6)-glucosamine structures, F-598 will selectively bind to acetylated β-(1→6)-glucosamine structures, *i.e.*, *N*-acetyl glucosamine.

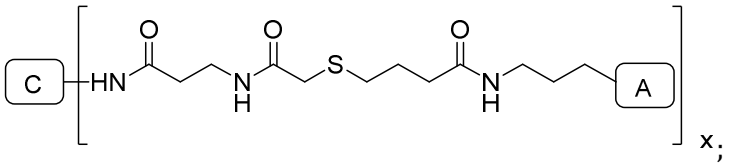
[0012] In one ~~embodiment~~example, this ~~invention~~disclosure provides for a vaccine against microbes comprising oligosaccharide β-(1→6)-glucosamine structures in their cell wall wherein said vaccine is represented by formula I:

$$(A\text{-}B)_x\text{-}C \quad I$$

where A comprises 3 to 12 β-(1→6)-glucosamine (carbohydrate ligand) groups or mixtures thereof wherein said oligosaccharide portion of the vaccine has the formula:



B is of the formula:



where A is as defined above and C is tetatus toxoid;

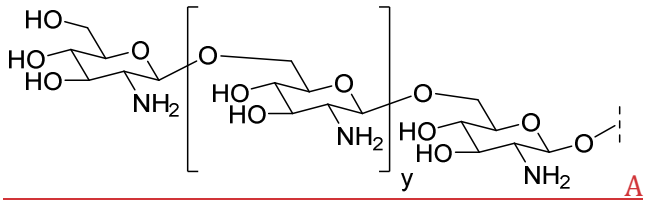
x is an integer from about 30 to about 39; and

y is an integer from 1 to 10.

[0012a] In one aspect, the present disclosure refers to use of a monoclonal antibody F-598 in the manufacture of a first medicament and use of a vaccine of formula I:

$$(A\text{-}B)_x\text{-}C \quad I$$

wherein A comprises 3 to 12 β-(1→6)-glucosamine (carbohydrate ligand) groups or mixtures thereof wherein said oligosaccharide portion of the vaccine is represented by formula A:



A

B is a linker ;

where A is as defined above and C is tetatus toxoid;

x is an integer from about 30 to about 39; and

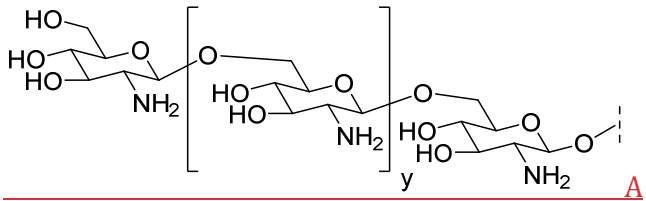
y is an integer from 1 to 10,

in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy for providing continuous protection against microbial infection in a patient.

[0012b] In another aspect, the present disclosure refers to use of a monoclonal antibody F-598 in the manufacture of a first medicament and use of a vaccine of formula I:

$$(A\text{-}B)_x\text{-}C \quad I$$

wherein A comprises 3 to 12 β-(1→6)-glucosamine (carbohydrate ligand) groups or mixtures thereof wherein said oligosaccharide portion of the vaccine is represented by formula A:



A

B is a linker ;

where A is as defined above and C is tetatus toxoid;

x is an integer from about 30 to about 39; and

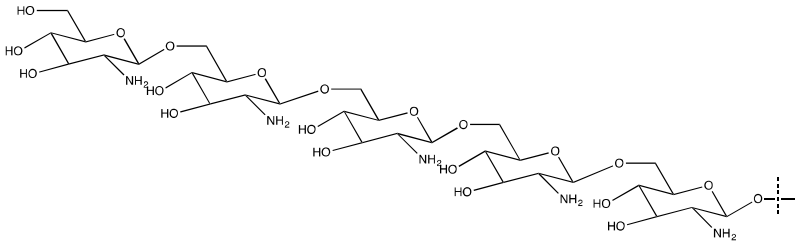
y is an integer from 1 to 10,

in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy for inhibiting biofilm formation in a patient.

[0013] In one ~~embodiment~~example, this ~~invention~~disclosure provides for a vaccine against microbes comprising oligosaccharide β-(1→6)-glucosamine structures in their cell wall wherein said vaccine is represented by formula II:

$$(A'\text{-}B)_x\text{-}C \quad II$$

where A′ is a penta-β-(1→6)-glucosamine (carbohydrate ligand) group of the formula:



and B, C and *x* are as defined above.

[0014] In one ~~embodiment~~example, this ~~invention~~disclosure provides for a pharmaceutical composition comprising a pharmaceutically acceptable diluent and an effective amount of the vaccine of formula I and/or formula II.

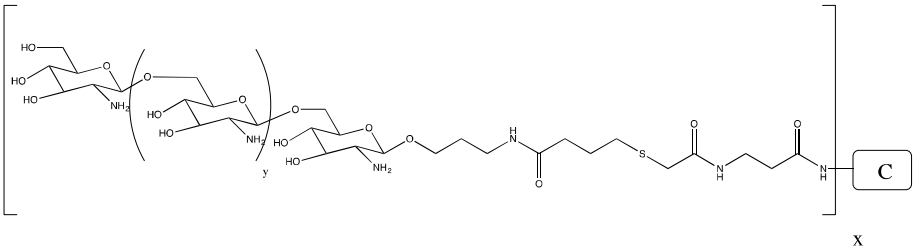
[0015] In one ~~embodiment~~example, this ~~invention~~disclosure provides for a method for providing immunity to a patient from microbes comprising oligosaccharide β-(1→6)-glucosamine groups in their cell wall which method comprises administering said vaccine of formula I and/or formula II to said patient.

[0016] In one ~~embodiment~~example, this ~~invention~~disclosure provides for a method for providing effective immunity to a patient from microbes comprising oligosaccharide β-(1→6)-glucosamine groups in their cell wall which method comprises administering the pharmaceutical composition of this ~~invention~~disclosure to said patient.

[0016a] In another aspect, the present disclosure refers to use of the vaccine disclosed herein in the manufacture of a medicament for providing effective immunity to a subject from microbes comprising oligosaccharide β-(1→6)-glucosamine groups in their cell wall.

[0016b] In yet another aspect, the present dislclosure refers to use of the vaccine disclosed herein in the manufacture of a medicament for providing effective protection to a subject against biofilm formed by microbes comprising oligosaccharide β-(1→6)-glucosamine groups in their cell wall.

[0017] Representative vaccines of this ~~invention~~disclosure are set forth in the table below:



Example	Y	C	*x*
A	2	Tetanus toxoid	30-39

B	3	Tetanus toxoid	35-39
C	2	Tetanus toxoid	35-39
D	3	Tetanus toxoid	30-39
E	3	Tetanus toxoid	30-35
F	4	Tetanus toxoid	35-39
G	8	Tetanus toxoid	35-39
H	10	Tetanus toxoid	35-39

[0018] In ~~embodiment~~examples, this ~~invention~~disclosure provides a method for providing immunity to a patient from microbes comprising oligosaccharide β-(1→6)-glucosamine groups in their cell wall which method comprises administering said vaccine of formula I and/or formula II to said patient concurrently with a monoclonal antibody F-598.

[0019] "Concurrently," as used herein, can include before or during administration of said vaccine. In some ~~embodiment~~examples, concurrent may include administration of the vaccine of formula I and/or formula II within about ±6 hours of administering F-598, or within ±4 hours, or within ±2 hours. In ~~embodiment~~examples, the two can be administered as part of the same bolus injection. Administration is "concurrent" so long as the patient is able to mount immune response based on each individual components. The order in which F-598 and the vaccine of formula I or II are administered is not critical. Concurrent administration can correspond to any period of time outside of 2 or 6 hours and still be concurrent so long as both sets of antibodies (from F-598 and those generated from vaccine) are effectively providing antibody coverage for their respective targets for an overlapping period of time.

[0020] Without being bound by theory, the methods disclosed herein are complementary and synergistic because of the respective selectivities of the F-598 antibody and the antibodies generated from the vaccines of formulas I and II. It has been found that F-598 binds with specificity to N-acetyl rich regions of cell wall PNAG structures of microbes as described in "Structural basis for antibody targeting of the broadly expressed microbial polysaccharide poly-*N*-acetyl glucosamine," *J. Biol. Chem.*

293(14) 5079-5089 (2018), which is incorporated herein by reference in its entirety. The vaccines of formulas I and II, provide selectivity for non-*N*-acetylated regions of PNAG cell wall structures. In some ~~embodiment~~examples, the presence of both populations of antibodies may minimize cross-reactivity and provide full protection against microbes having PNAG-bearing cell wall structures.

[0021] In some ~~embodiment~~examples, F-598 is co-administered during the entire treatment period.

[0022] In some ~~embodiment~~examples, F-598 is co-administered only up until a point where sufficient antibody titer is produced by the vaccines of formula I and/or formula II to effectively treat the patient. After the period in which there is such sufficient antibody produced by the vaccine, administration of F-598 may be terminated.

[0023] In some ~~embodiment~~examples, F-598 may be terminated immediately after there is a measured sufficient titer of vaccine generated antibody. In ~~embodiment~~examples, F-598 may be terminated one week after there is a measured sufficient titer of vaccine generated antibody. In ~~embodiment~~examples, F-598 may be terminated two weeks after there is a measured sufficient titer of vaccine generated antibody. In ~~embodiment~~examples, F-598 may be terminated one month after there is a measured sufficient titer of vaccine generated antibody. Those skilled in the art will appreciate that the exact period where it may be determined by the specific conditions/state of the patient.

[0024] In some ~~embodiment~~examples, administering said vaccine of formula I and/or formula II may comprise a regimen of one to three administrations. For example, for some patients a single administration may be sufficient. For some patients two administrations may be needed. For some patients, three administrations may be needed. Among factors that may contribute to the number of administrations may be the age and condition of the patient. Very young patients with newly forming immune systems may require more than one administration. Similarly, elderly patients with immune systems in decline may require more than one administration.

[0025] In some ~~embodiment~~examples, a treatment regimen includes monitoring of the patient for depletion of F-598 and/or the need for additional administrations of vaccine based on antibody titers. For example, a burn victim may require additional dosing of F-

598 due to secretion of antibody at the site of the wound. Accordingly, in some ~~embodiment~~examples, the serum concentration of antibodies is evaluated periodically in order to maintain proper titer throughout the entire treatment regimen.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] Figure 1 illustrates the ^{1}H NMR for compound 17 (as described below).

[0027] Figure 2 illustrates the ^{13}C NMR for compound 17.

DETAILED DESCRIPTION OF THE INVENTION

[0028] This invention provides for antimicrobial vaccines comprising oligosaccharide β-(1→6)-glucosamine groups having from 3 to 12 glucosamine units linked to an immunogenic protein.

[0029] Prior to describing this invention in more detail, the following terms will first be defined. If a term used herein is not defined, it has its generally accepted scientific or medical meaning.

[0030] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

[0031] "Optional" or "optionally" means that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where the event or circumstance occurs and instances where it does not.

[0032] The term "about" when used before a numerical designation, *e.g.*, temperature, time, amount, concentration, and such other, including a range, indicates approximations which may vary by (+) or (-) 10%, 5%, 1%, or any subrange or subvalue there between. Preferably, the term "about" when used with regard to a dose amount means that the dose may vary by +/- 10%.

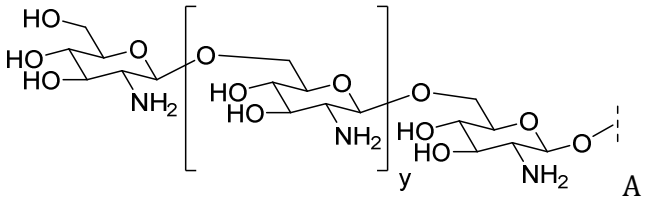
[0033] "Comprising" or "comprises" is intended to mean that the compositions and methods include the recited elements, but not excluding others. "Consisting

WHAT IS CLAIMED IS:

1. Use~~A method of providing continuous protection against microbial infection, which method comprises administering to said patient a therapeutically effective amount~~ of a monoclonal antibody F-598 in the manufacture of a first medicament and use of a vaccine of formula I:

$$(A\text{-}B)_x\text{-}C \quad I$$

wherein A comprises 3 to 12 β-(1→6)-glucosamine (carbohydrate ligand) groups or mixtures thereof wherein said oligosaccharide portion of the vaccine is represented by formula A:



B is a linker ;

where A is as defined above and C is tetatus toxoid;

x is an integer from about 30 to about 39; and

y is an integer from 1 to 10~~.~~,

in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy for providing continuous protection against microbial infection in a patient.

2. The ~~method~~ use of claim 1, wherein the vaccine of formula I is to be administered concurrently with F-598.

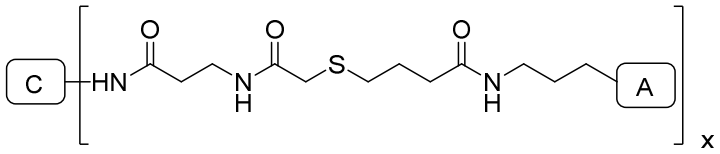
3. The ~~method~~ use of claim 1, wherein the vaccine of formula I is to be administered within about 6 hours of administering F-598.

4. The ~~method~~ use of claim 1, wherein the vaccine of formula I is to be administered within about 4 hours of administering F-598.

5. The ~~method~~ use of claim 1, wherein the vaccine of formula I is to be administered within about 2 hours of administering F-598.

6. The ~~method~~ use of claim 1, wherein F-598 is to be co-administered during an entire treatment period.

7. The ~~method~~ use of claim 1, wherein the linker is represented by the formula:



where A and C are not included within the linker.

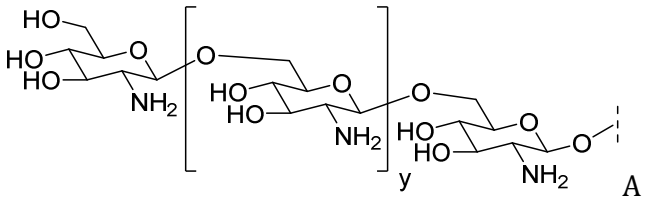
8. The ~~method~~ use of claim 1, wherein F-598 is to be co-administered up until a point where sufficient antibody titer is produced by the vaccine of formula I to effectively treat the patient.

9. Use of the vaccine of claim 7 in the manufacture of a medicament ~~A method~~ for providing effective immunity to a subject from microbes comprising oligosaccharide β-(1→6)-glucosamine groups in their cell wall ~~which method comprises administering the vaccine of claim 7 to said subject~~.

10. Use ~~A method of inhibiting biofilm formation comprising administering to a patient a therapeutically effective amount~~ of a monoclonal antibody F-598 in the manufacture of a first medicament and use of a vaccine of formula I:

$$(A\text{-}B)_x\text{-}C \quad I$$

wherein A comprises 3 to 12 β-(1→6)-glucosamine (carbohydrate ligand) groups or mixtures thereof wherein said oligosaccharide portion of the vaccine is represented by formula A:



B is a linker ;

where A is as defined above and C is tetatus toxoid;

x is an integer from about 30 to about 39; and

y is an integer from 1 to 10~~.~~,

in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy for inhibiting biofilm formation in a patient.

11. The ~~method~~ use of claim 10, wherein the vaccine of formula I is to be administered concurrently with F-598.

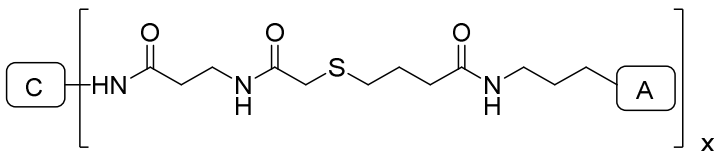
12. The ~~method~~ use of claim 10, wherein the vaccine of formula I is to be administered within about 6 hours of administering F-598.

13. The ~~method~~ use of claim 10, wherein the vaccine of formula I is to be administered within about 4 hours of administering F-598.

14. The ~~method~~ use of claim 10, wherein the vaccine of formula I is to be administered within about 2 hours of administering F-598.

15. The ~~method~~ use of claim 10, wherein F-598 is to be co-administered during an entire treatment period.

16. The ~~method~~ use of claim 10, wherein the linker is represented by the formula:



where A and C are not included within the linker.

17. The ~~method~~ use of claim 10, wherein F-598 is to be co-administered up until a point where sufficient antibody titer is produced by the vaccine of formula I to effectively treat the patient.

18. Use of the vaccine of claim 16 in the manufacture of a medicament ~~A method~~ for providing effective protection to a subject against biofilm formed by microbes comprising oligosaccharide β-(1→6)-glucosamine groups in their cell wall ~~which method comprises administering the vaccine of claim 16 to said subject~~.