SUMMARY

Provided herein are adjuvants, vaccine formulations comprising the adjuvants, methods of making the adjuvants and methods of using the adjuvants and vaccine formulations. In particular, chitosan and an antigen may be cross-linked using an aldehyde.

In one aspect, there is provided an adjuvant composition comprising a carbohydrate linked to chitosan to form a Schiff base and wherein the carbohydrate is mannose.

In one aspect, there is provided an adjuvant composition comprising between 0.5% and 2% of an aldehyde cross-linked chitosan and Tris-HCl to quench free aldehydes.

In one aspect, there is provided a vaccine formulation comprising the adjuvant composition as disclosed herein and an antigen, wherein the antigen comprises a microbe.

In one aspect, there is provided a method of making the composition as disclosed herein, comprising dissolving chitosan in a solution of acetic acid, adding an antigen to the dissolved chitosan; combining the dissolved chitosan and antigen with an aldehyde such that the final concentration of the aldehyde is between 0.02% and 0.5% and adding Tris-HCl to quench free aldehydes.

In one aspect, there is provided a use of the vaccine formulation as disclosed herein in the manufacture of a medicament for enhancing the immune response of a subject to an antigen.

In one ~~aspect~~embodiment, a composition comprising 0.5% to 2% of an aldehyde cross-linked chitosan and an antigen is provided. The final concentration of aldehyde in a vaccine composition is less than 0.5%.

In another ~~aspect~~embodiment, an adjuvant composition comprising a carbohydrate linked to chitosan to form a Schiff base is provided. The adjuvant may be combined with an antigen. The carbohydrate may be mannose.

In yet another ~~aspect~~embodiment, vaccine formulations are provided. Vaccine formulations may include the adjuvants provided herein and an antigen. The antigens may be proteins or microbial in nature, suitable microbes include bacteria, yeast, or other fungi, eukaryotic parasites and viruses and may be attenuated, recombinant, killed or otherwise inactivated.

In still another ~~aspect~~embodiment, methods of making the adjuvants and vaccine compositions are provided herein. The chitosan is dissolved in a solution of acetic acid, and an antigen is added to the dissolved chitosan. Finally the antigen and chitosan are combined with

an aldehyde such that the final concentration of the aldehyde is between 0.02% and 0.5%. Tris may be added to the adjuvant to quench free aldehydes and result in a more stable adjuvant.

In a still further ~~aspect~~embodiment, methods of enhancing the immune response of a subject to an antigen are also provided. The methods include administering a vaccine formulation comprising an antigen and a chitosan-based adjuvant disclosed herein to the subject. The chitosan-based adjuvant may be an aldehyde cross-linked chitosan or a carbohydrate-linked chitosan.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing the anti-β-galactosidase IgG antibody response in turkeys following primary vaccination and boost with the indicated vaccine-adjuvant formulations. Different letters indicate significant differences ($p \leq 0.05$).

Figure 2 is a graph showing the *Clostridium septicum* IgG antibody response in turkeys following primary vaccination and boost with the indicated vaccine-adjuvant formulations. Different letters indicate significant differences ($p \leq 0.05$).

We claim:

1. An adjuvant composition comprising a carbohydrate linked to chitosan to form a Schiff base and wherein the carbohydrate is mannose.

~~2. The composition of claim 1, wherein the carbohydrate is selected from mannose, mannobiose, glucose, galactose, or fructose.~~

~~3. The composition of claim 2, wherein the carbohydrate is mannose.~~

~~4.~~2. The composition of ~~any one of~~ claims 1-~~3~~, wherein the Schiff base is not reduced.

~~5.~~3. The composition of ~~any one of~~ claims 1-~~3~~, wherein the Schiff base is reduced.

~~6.~~4. An adjuvant composition comprising between 0.5% and 2% of an aldehyde cross-linked chitosan and Tris-HCl to quench free aldehydes.

~~7.~~5. The composition of claim ~~4~~6, wherein the chitosan is cross-linked with formaldehyde.

~~8. The composition of claim 6 or 7, further comprising Tris-HCl to quench free aldehydes.~~

~~9.~~6. The composition of any one of claims 1-~~5~~8, further comprising an enhancing molecule.

~~10.~~7. The composition of claim ~~6~~9, wherein the enhancing molecule is saponin, toll-like receptors, the B subunit of a bacterial toxin, bacterial toxins, CpG motifs, liposomes or monophosphoryl lipid A.

~~11.~~8. The composition of claim ~~6~~9, wherein the enhancing molecule is tetanus toxoid, cholera toxin B subunit, heat labile enterotoxin B subunit, or tripolyphosphate.

~~12.~~9. A vaccine formulation comprising the adjuvant composition of any one of claims 1-~~8~~11 and an antigen, wherein the antigen comprises a microbe.

~~13. The vaccine of claim 12, wherein the antigen is a protein.~~

~~14. The vaccine of claim 13, wherein the protein is *Influenza* M2e, Hemaglutinin, Neuraminidase, or nuclear proteins; *Eimeria* TRAP or MPP; *Clostridium* sialidase, SagA, alpha-toxin, NetB toxin, or iron transport protein.~~

~~15. The vaccine of claim 12, wherein the antigen comprises a microbe.~~

~~16.~~10. The vaccine of claim ~~9~~15, wherein the microbe is *Salmonella, Escherichia, Shigella, Bordetella, Clostridium, Mycoplasma, Staphylococcus, Streptococcus, Bacillus, Influenza*, or *Eimeria*.

~~17.~~11. The vaccine of claim ~~9~~15 or 10~~16~~, wherein the microbe is inactivated or killed.

~~18.~~12. The vaccine of claim 11~~17~~, wherein the microbe is killed using formaldehyde, glutaraldehyde or formalin.

~~19.~~13. A method of making the composition of any one of claims 4-5~~6~~-8, comprising dissolving chitosan in a solution of acetic acid, adding an antigen to the dissolved chitosan; ~~and~~ combining the dissolved chitosan and antigen with an aldehyde such that the final concentration of the aldehyde is between 0.02% and 0.5% and adding Tris-HCl to quench free aldehydes.

~~20.~~14. The method of claim 13~~19~~, wherein the antigen is a protein.

~~21.~~15. The method of claim 13~~19~~, wherein the antigen is microbial vaccine.

~~22. The method of any one of claims 19-21, further comprising adding Tris-HCl to quench free aldehydes.~~

~~23.~~16. The method of any one of claims 13-15~~19-22~~, wherein the aldehyde is added at a final concentration of 0.2%.

~~24.~~17. ~~A method of enhancing the immune response of a subject to an antigen comprising administering~~Use of the vaccine formulation of any one of claims 9-12~~12-18~~ in the manufacture of a medicament for enhancing the immune response of a subject to an antigen~~to the subject~~.

~~25.~~18. The use~~method~~ of claim 17~~24~~, wherein the immune response includes an enhanced antibody response as compared to administering the~~a~~ vaccine without an adjuvant.

~~26.~~19. The use~~method~~ of claim 18~~25~~, wherein the enhanced antibody response is an enhanced secretory IgA antibody response ~~is enhanced~~ as compared to administering the antigen~~a vaccine~~ without an adjuvant.

~~27. The method of claim 26, wherein the IgA response after administration of the vaccine is increased at least two-fold relative to the IgA response of a subject administered a control vector.~~

~~28.~~20. The use~~method~~ of any one of claims 17-19~~24-27~~, wherein the subject is a mammal or poultry.

29. The ~~method~~ use of any one of claims ~~24-28~~17-20, wherein the route of administration is subcutaneous or oral.

30. The use of any one of claims 17-21, wherein the vaccine formulation is administered in the food or drinking water.