antibody may be specific for an epitope on the surface of the microorganism. The epitope may be conserved. The CD40 agonistic antibody may be specific for chicken CD40 and may include or consist of SEQ ID NO: 2 and SEQ ID NO: 4 or SEQ ID NO: 14. Alternatively the CD40 agonistic antibody may include the CDR regions of SEQ ID NOs: 5-10 or the CDR regions of SEQ ID NOs: 17-22. The killed pathogen may be Influenza or a bacterium or a bacterial cell surface fragment.

The adjuvant composition can be combined with the microorganism via interaction with the second antibody to produce a vaccine. The serotype of the microorganism may be unknown. The microorganism need not be purified to interact with the second antibody. The microorganism may be killed or inactivated prior to binding to the second antibody to form a complex.

In another aspect, a vaccine comprising the adjuvant composition as disclosed herein and further comprising the microorganism, wherein the adjuvant composition is specifically bound to the microorganism is provided.

In another aspect, a CD40 agonistic antibody or a portion thereof comprising at least an F(ab) region is provided. The CD40 agonistic antibody or portion thereof is selected from the following: an antibody comprised of SEQ ID NO: 2 and SEQ ID NO: 4; an antibody comprising SEQ ID NO: 14; an antibody or portion thereof comprising a heavy chain variable (V_H) region and a light chain variable (V_L) region, wherein the heavy chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 5, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 7 and wherein the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 8, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 9, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 10; and an antibody or portion thereof comprising a heavy chain variable (V_H) region and a light chain variable (V_L) region, wherein the heavy chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 20, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 21, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 22 and wherein the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 17, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 18, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 19.

In a further aspect, the CD40 agonistic antibodies may be used to generate a vaccine. In the vaccine, the CD40 agonistic antibody is linked via a linker moiety to an antigen. The antigen

may be a peptide. The vaccines may be comprised within an alginate sphere for administration in the food or drinking water.

In a further aspect, methods of enhancing an immune response in a subject are provided. The methods include administering the vaccines or compositions provided herein to the subject in an amount effective to enhance the immune response to the antigen or microorganism. The vaccine or composition may be administered mucosally, may induce both IgG and IgA, in particular sIgA, and induces a rapid response within about 7 days.

In a still further aspect, constructs for production of a vaccine composition. The construct includes a first polynucleotide encoding an anti-CD40 agonistic antibody heavy chain comprising SEQ ID NO: 5, 6, and 7 or SEQ ID NO: 20, 21 and 22 and an anti-CD40 agonistic antibody light chain comprising SEQ ID NO: 8, 9, and 10 or SEQ ID NO: 17, 18 and 19. The polynucleotide is operably connected to a promoter to allow for expression of the anti-CD40 agonistic antibody. The construct may further include a second polynucleotide encoding an antigen and the two polynucleotides may be linked in frame to form a fusion protein when expressed.

In a still further aspect, methods of epitope mapping a polypeptide are provided. Labeled peptides of 8-20 amino acids from the polypeptide are generated and attached to a labeled CD40 antibody via a linker moiety to create a CD40 antibody-peptide complex. The CD40 antibody-peptide complex was administered to a subject and after a period of time that may be as short as 5-7 days sera was collected from the subject and tested for the presence of antibodies able to recognize the polypeptide. Peptides capable of producing antibodies to the polypeptide were identified as antigenic epitopes. These identified antigenic epitopes may be used to develop a vaccine.

In another aspect, a pharmaceutical composition comprising the vaccine as disclosed herein and a pharmaceutically acceptable carrier is provided.

In another aspect, the use of the vaccine as disclosed herein or the composition as disclosed herein in the manufacture of a medicament to enhance the immune response to the antigen or microorganism in the vaccine or composition is provided.

In another aspect, the cell comprising the construct as disclosed herein is provided.

In another aspect, a method of epitope mapping a polypeptide comprising:

a. Generating labeled peptides of 8-20 amino acids from the polypeptide;

b. Attaching the labeled peptides to a labeled CD40 antibody via a linker moiety to create a CD40 antibody-peptide complex;

c. Administering the CD40 antibody-peptide complex to a subject;

d. Collecting sera from the subject;

e. Testing the sera for the presence of antibodies able to recognize the polypeptide; and

f. Identifying the peptides capable of producing antibodies to the polypeptide as antigenic epitopes

is provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation showing the preparation of antibody-peptide complex based on biotin-streptavidin interaction. Fig. 1A shows that biotinylation was limited to the carbohydrate groups on the Fc region of MIg, hence did not interfere with antigen-antibody interaction. Fig. 1B shows that streptavidin (SA) was used for controlled complexing of biotinylated peptide with biotinylated MIg. Mab 2C5 in the 2C5-SA-peptide complex retained its biological function as demonstrated by ELISA.

CLAIMS

We claim:

1. An adjuvant composition comprising at least one first CD40 agonistic antibody or portion thereof comprising at least two F(ab) regions capable of specifically binding CD40 and inducing CD40 signaling, at least one second antibody or portion thereof comprising at least two F(ab) regions capable of specifically binding a microorganism, at least one label attached to the at least one first CD40 agonistic antibody or portion thereof and the at least one second antibody or portion thereof, and a linker moiety capable of specifically binding to the labels, wherein the at least one first CD40 agonistic antibody and the at least one second antibody are bound to the linker moiety to form a complex.

~~2. The adjuvant composition of claim 1, wherein the linker moiety binds to the labels on the first CD40 agonistic antibody and the second antibody non-covalently.~~

~~3.~~2. The adjuvant composition of any one of the preceding claims, wherein two or more of the first CD40 agonistic antibody and two or more of the second antibody are bound to the linker moiety to form the~~a~~ complex.

~~4. The adjuvant composition of any one of the preceding claims, wherein the label is covalently attached to each of the antibodies.~~

~~5.~~3. The adjuvant composition of any one of the preceding claims, wherein the label on each of the first CD40 agonistic antibody and the second antibody is biotin.

~~6.~~4. The adjuvant composition of any one of the preceding claims, wherein the linker moiety is avidin or streptavidin.

~~7.~~5. The adjuvant composition of any one of the preceding claims, wherein the microorganism to which the second antibody specifically binds is a bacterium or a virus.

~~8.~~6. The adjuvant composition of claim ~~5~~7, wherein the second antibody specifically binds a microorganism selected from the group consisting of influenza virus, *Salmonella, Clostridium, Campylobacter, Escherichia, Shigella, Helicobacter, Vibrio, Plesiomonas, Edwardia, Clostridia, Klebsiella, Staphylococcus, Streptococcus, Aeromonas*, Foot and Mouth virus, porcine epidemic diarrhea virus (PEDv), and Porcine reproductive and respiratory syndrome virus (PRRSV).

~~9.~~7. The adjuvant composition of claim ~~6~~8, wherein the second antibody binds Influenza M2e.

~~10.~~8. The adjuvant composition of any one of the preceding claims, wherein the first CD40 agonistic antibody or portion thereof is selected from the group consisting of at least one of:

a. An antibody comprised of SEQ ID NO: 2 and SEQ ID NO: 4 (2C5);

b. An antibody comprised of SEQ ID NO: 14 (DAG1);

c. An antibody or portion thereof comprising a heavy chain variable (V_H) region and a light chain variable (V_L) region, wherein the heavy chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 5, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 7 and wherein the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 8, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 9, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 10; and

d. An antibody or portion thereof comprising a heavy chain variable (V_H) region and a light chain variable (V_L) region, wherein the heavy chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 20, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 21, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 22 and wherein the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 17, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 18, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 19.

~~11. The adjuvant composition of any one of the preceding claims, wherein the first CD40 agonistic antibody is specific for a CD40 polypeptide from a species selected from the group consisting of chicken, turkey, bovine, porcine, fish, goats, sheep, dogs, cats and other domesticated animals.~~

~~12.~~9. A vaccine comprising the adjuvant composition of any one of the preceding claims and further comprising the microorganism, wherein the adjuvant composition is specifically bound to the microorganism.

~~13. The vaccine of claim 12, wherein the microorganism is an influenza virus.~~

~~14.~~10. The vaccine of ~~any one of~~ claim~~s~~ 9~~12-13~~, wherein the microorganism is killed or inactivated.

~~15.~~11. The vaccine of any one of claims 9-10~~12-14~~, wherein the vaccine is comprised within alginate spheres.

~~16. The vaccine of any one of claims 12-15 or the adjuvant composition of any one of claims 1-11, wherein the serotype of the microorganism is unknown.~~

~~17.~~12. A CD40 agonistic antibody or a portion thereof comprising at least an F(ab) region, the CD40 agonistic antibody or portion thereof selected from the group consisting of at least one of:

a. An antibody comprised of SEQ ID NO: 2 and SEQ ID NO: 4 (2C5);

b. An antibody comprising SEQ ID NO: 14 (DAG1);

c. An antibody or portion thereof comprising a heavy chain variable (V_H) region and a light chain variable (V_L) region, wherein the heavy chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 5, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 7 and wherein the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 8, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 9, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 10 (2C5); and

d. An antibody or portion thereof comprising a heavy chain variable (V_H) region and a light chain variable (V_L) region, wherein the heavy chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 20, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 21, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 22 and wherein the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 17, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 18, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 19 (DAG1).

~~18.~~13. A vaccine comprising the CD40 agonistic antibody or portion thereof of claim 12~~17~~ linked to an antigen by a linker moiety.

~~19.~~14. The vaccine of claim 13~~18~~, wherein the linker moiety is selected from the group consisting of a peptide and streptavidin, and wherein when the linker moiety is streptavidin the CD40 agonistic antibody is biotinylated and the antigen is biotinylated such that the linker moiety is capable of linking the CD40 agonistic antibody to the antigen.

~~20. The vaccine of claim 18, wherein the linker moiety is streptavidin, the CD40 agonistic antibody is biotinylated and the antigen is biotinylated such that the linker moiety is capable of linking the CD40 agonistic antibody to the antigen.~~

~~21.~~15. The vaccine of any one of claims 13-14~~18-20~~, wherein the antigen is selected from the group consisting of a vaccine, an influenza virus, a microorganism, a peptide, *Salmonella, Clostridium perfringens, Campylobacter, Escherichia, Shigella, Helicobacter, Vibrio, Plesiomonas, Edwardia, Clostridia, Klebsiella, Staphylococcus, Streptococcus, Aeromonas*, Foot and Mouth virus, porcine epidemic diarrhea virus (PEDv), and Porcine reproductive and respiratory syndrome virus (PRRSV).

~~22. The vaccine of any one of claims 18-20, wherein the antigen is an influenza virus.~~

~~23. The vaccine of any one of claims 18-20, wherein the antigen is a microorganism.~~

~~24. The vaccine of claim 23, wherein the microorganism is selected from the group consisting of *Salmonella, Clostridium perfringens, Campylobacter, Escherichia, Shigella, Helicobacter, Vibrio, Plesiomonas, Edwardia, Clostridia, Klebsiella, Staphylococcus, Streptococcus, Aeromonas*, Foot and Mouth virus, porcine epidemic diarrhea virus (PEDv), and Porcine reproductive and respiratory syndrome virus (PRRSV).~~

~~25. The vaccine of any one of claims 18-20, wherein the antigen is a peptide.~~

~~26.~~16. The vaccine of any one of claims 13-15~~18-25~~, wherein the vaccine is comprised within alginate spheres.

~~27.~~17. A pharmaceutical composition comprising the vaccine of any one of claims 9-11~~12-16~~ or~~and~~ 13-16~~18-26~~ and a pharmaceutically acceptable carrier.

~~28.~~18. A use of~~method of enhancing an immune response in a subject comprising administering~~ the vaccine of any one of claims 9-11~~12-16~~ or~~and~~ 13-16~~18-26~~ or the composition of claim 17~~27~~ in the manufacture of a medicament ~~to the subject in an amount effective~~ to enhance the immune response to the antigen or *microorganism* in the vaccine or composition.

~~29.~~19. The use~~method~~ of claim 18~~28~~, wherein administration is via a route selected from the group consisting of mucosal, oral, cloacal, nasal, ocular and~~or~~ subcutaneous routes.

~~30. The method of claim 29, wherein the mucosal route is oral, cloacal, nasal, or ocular.~~

~~31.~~20. The use~~method~~ of claim 18~~28~~, wherein the vaccine or composition is administered in the food or drinking water.

~~32. The method of any one of claims 28-31, wherein the IgG and IgA response is enhanced.~~

~~33.~~21. The use~~method~~ of any one of claims 18-20~~28-32~~, wherein the immune response is enhanced within 7 days of administration and wherein a single administration is sufficient to induce the immune response.

~~34. The method of any one of claims 28-33, wherein a single administration is sufficient to induce the immune response.~~

~~35. The method of any one of claims 28-34, wherein the CD40 antibody is selected to be specific for the CD40 of the subject's taxonomic species, genus or family.~~

~~36.~~22. The use~~method~~ of any one of claims 18-21~~35~~, wherein the CD40 antibody is specific for chicken CD40 and the subject is a chicken.

~~37.~~23. A construct comprising a first polynucleotide encoding a CD40 agonistic antibody heavy chain comprising SEQ ID NO: 5, 6, and 7 or SEQ ID NO: 20, 21 and 22 and a CD40 agonistic antibody light chain comprising SEQ ID NO: 8, 9, and 10 or SEQ ID NO: 17, 18 and 19 and wherein the first polynucleotide is operably connected to a promoter to allow for expression of the CD40 agonistic antibody.

~~38.~~24. The construct of claim 23~~37~~, further comprising a polynucleotide encoding a secretory signal such that the CD40 agonistic antibody can be secreted from cells.

~~39.~~25. The construct of claim 23~~37~~ or 24~~38~~, further comprising a multi-cloning site to enable incorporation of additional polynucleotides encoding antigens in the construct.

~~40.~~26. The construct of claim 23~~37~~ or 24~~38~~, further comprising a second polynucleotide encoding an antigen.

~~41.~~27. The construct of claim 26~~40~~, wherein the antigen is selected from SEQ ID NOs: 26-53 or 57-83.

~~42.~~28. The construct of claim 26~~40~~ or 27~~41~~, wherein the second polynucleotide encoding the antigen is linked in frame to the polynucleotide encoding the CD40 agonistic antibody.

~~43.~~29. A cell comprising the construct of any one of claims 23-28~~37-42~~.

~~44.~~30. The cell of claim 29~~43~~, wherein the cell is a plant, algal, bacterial, insect or yeast cell.

~~45.~~31. A method of epitope mapping a polypeptide comprising:

a. Generating labeled peptides of 8-20 amino acids from the polypeptide;

b. Attaching the labeled peptides to a labeled CD40 antibody via a linker moiety to create a CD40 antibody-peptide complex;

c. Administering the CD40 antibody-peptide complex to a subject;

d. Collecting sera from the subject;

e. Testing the sera for the presence of antibodies able to recognize the polypeptide; and

f. Identifying the peptides capable of producing antibodies to the polypeptide as antigenic epitopes.

~~46.~~32. The method of claim 3145, wherein the label is biotin.

~~47.~~33. The method of any one of claims 31-3245-46, wherein the linker moiety is streptavidin.

~~48.~~34. The method of any one of claims 31-3345-47, wherein the testing step includes at least one of an ELISA assay, Western blot, immunofluorescence or a neutralization assay.

~~49.~~35. The method of any one of claims 31-3445-48, further comprising developing a vaccine including the identified antigenic epitopes.