or the receptor binding domain (RBD) of the S protein in advance of infection. However, this approach poses a great challenge in that the S protein is not well conserved even between SARS-CoV-1 and SARS-CoV-2 strains. In particular, small amino acid changes that occur among variants often result in conformational changes to the S protein that may significantly reduce the effectiveness of nAb elicited by the specific S protein of the COVID-19 vaccine.

[0006] Continued vaccine development targeting only the betacoronavirus S protein is therefore contemplated to follow the path of seasonal influenza vaccines. This means that the continual emergence of variants will likely require development and production of new vaccines on a periodic basis. Although annual production of betacoronavirus vaccines may be technically feasible, global vaccination efforts involving annual administration of new vaccines are economically and logistically impractical. The problems posted by annual administration of new vaccines present especially undue burdens for low- and middle-income countries.

[0007] Accordingly, there is a need in the art for betacoronavirus vaccines that safely induce a long-lived, immune response that is broadly reactive against SARS-CoV-2 variants. Preferably the long-lived, immune response is broadly reactive with other betacoronaviruses, which cause disease in humans. There is also a need in the art for influenza virus vaccines that are safe and effective in inducing a broadly reactive immune response against influenza A and/or influenza B viruses.

SUMMARY

[0008] The present disclosure relates to the use of nucleoproteins (also referred to herein as nucleocapsid proteins) from betacoronaviruses as a vaccine antigen to induce cellular immune responses that are broadly reactive with betacoronavirus variants. In some examples, a temperature-controllable, self-replicating RNA (referred to herein as srRNAts and c-srRNA) vaccine platform is utilized. The c-srRNA vaccine platform is advantageous for induction of a potent cellular immune response after intradermal administration. In some examples, a nucleoprotein from SARS-CoV-2 is expressed in host cells to address infection by both SARS-CoV-2 and SARS-CoV-1, as well as variants thereof. In some examples, a nucleoprotein from a coronavirus is fused with a signal peptide of the human CD5 antigen and expressed in host cells to enhance the cellular immune response elicited against the coronavirus. In some

examples, a nucleoprotein from a first coronavirus is fused to a nucleoprotein from a second coronavirus, which is different from the first coronavirus. In some examples, the fusion protein comprises a tandem array of two or three coronavirus nucleoproteins. In a subset of these examples, the fusion protein comprises a SARS-CoV-2 nucleoprotein and a MERS-CoV nucleoprotein. In some examples, the fusion protein further comprises a coronavirus spike protein or fragment thereof. In this way, a more broadly reactive coronavirus-specific immune response is stimulated.

[0009] The present disclosure also relates to the use of nucleoproteins (also referred to herein as nucleocapsid proteins) from influenza viruses as a vaccine antigen to induce cellular immune responses that are broadly reactive with influenza A and/or influenza B viruses, which rapidly change over time as a consequence of antigen drift and antigen shift. In some examples, a temperature-controllable, self-replicating RNA vaccine platform is utilized. The c-srRNA vaccine platform is advantageous for induction of a potent cellular immune response after intradermal administration. In some examples, a nucleoprotein from one subtype of influenza A (FluA) virus is expressed in host cells to address infection by the same and different subtypes of FluA. In some examples, a nucleoprotein from one lineage of influenza B (FluB) virus is expressed in host cells to address infection by the same and different lineages of FluB. In some examples, a nucleoprotein from an influenza virus is fused with a signal peptide of the human CD5 antigen and expressed in host cells to enhance the cellular immune response elicited against the influenza virus. In some examples, a nucleoprotein from a FluA virus is fused to a nucleoprotein from a FluB virus. In some examples, the fusion protein comprises a tandem array of two or three nucleoproteins from one or more strains of FluA and/or one or more lineages of FluB. In some examples, the fusion protein further comprises an influenza hemagglutinin or fragment thereof. In this way, a more broadly reactive influenza-specific immune response is stimulated.

[0010] The present disclosure also relates to the use of nucleoproteins (also referred to herein as nucleocapsid proteins) from ebolaviruses as a vaccine antigen to induce cellular immune responses that are broadly reactive with two, three or four species of ebolavirus that infect humans. In some examples, a temperature-controllable, self-replicating RNA vaccine platform is utilized. The c-srRNA vaccine platform is advantageous for induction of a potent

cellular immune response after intradermal administration. In some examples, a nucleoprotein from an ebolavirus is fused with a signal peptide of the human CD5 antigen and expressed in host cells to enhance the cellular immune response elicited against the ebolavirus. In some examples, a nucleoprotein from a first ebolavirus species is fused to a nucleoprotein from a second ebolavirus species, which is optionally fused to a nucleoprotein of a third ebolavirus species, which is optionally fused to a nucleoprotein of a fourth ebolavirus species. In some examples, the fusion protein comprises a tandem array of two, three or four nucleoproteins or fragments thereof from two or more species of ebolavirus. In some examples, the fusion protein further comprises an ebolavirus envelope glycoprotein or fragment thereof. In this way, a more broadly reactive ebolavirus-specific immune response is stimulated.

[0011] Among other examples, the present disclosure provides compositions comprising an excipient and a temperature-controllable, self-replicating RNA. In some examples, the composition comprises a chitosan. In some examples, the chitosan is a low molecular weight (about 3-5 kDa) chitosan oligosaccharide, such as chitosan oligosaccharide lactate. In some examples, the composition does not comprise liposomes or lipid nanoparticles.

[0011a] In one aspect, the present disclosure refers to a composition suitable for stimulating an immune response against two or more viruses in a mammalian subject, comprising an excipient, and a messenger RNA (mRNA) comprising an open reading frame (ORF) encoding a fusion protein, wherein the ORF comprises from 5' to 3': (i) a nucleotide sequence encoding a mammalian signal peptide; and (ii) a nucleotide sequence encoding a first nucleocapsid protein of a first virus and a second nucleocapsid protein of a second virus.

[0011b] In one aspect, the present disclosure refers to a composition suitable for stimulating an immune response against a virus in a mammalian subject, comprising an excipient, and a messenger RNA (mRNA) comprising an open reading frame (ORF) encoding a fusion protein, wherein the ORF comprises from 5' to 3': (i) a nucleotide sequence encoding a mammalian signal peptide; (ii) a nucleotide sequence encoding a first viral antigen or fragment thereof of a first virus; and (iii) a nucleotide sequence encoding a second viral antigen or fragment thereof of the first virus or a second virus, wherein the first viral antigen is a nucleocapsid protein and the second viral antigen is a surface protein, or the first viral antigen is a surface protein and the second viral antigen is a nucleocapsid protein.

[0011c] In one aspect, the present disclosure refers to a composition suitable for stimulating an immune response against two or more viruses in a mammalian subject, comprising an excipient, and a messenger RNA (mRNA) comprising an open reading frame (ORF) encoding a fusion protein, wherein the ORF comprises from 5' to 3': (i) a nucleotide sequence encoding a mammalian signal peptide; (ii) a nucleotide sequence encoding a first viral antigen or fragment thereof of a first virus; (iii) a nucleotide sequence encoding a second viral antigen or fragment thereof of the first virus; (iv) a nucleotide sequence encoding a third viral antigen or fragment thereof of a second virus; (iii) a nucleotide sequence encoding a fourth viral antigen or fragment thereof of the second virus, wherein the first viral antigen is a first nucleocapsid protein and the second viral antigen is a first surface protein, or the first viral antigen is a first surface protein and the second viral antigen is a first nucleocapsid protein, and wherein the third viral antigen is a second nucleocapsid protein and the fourth viral antigen is a second surface protein, or the third viral antigen is a second surface protein and the fourth viral antigen is a second nucleocapsid protein.

[0011d] In one aspect, the present disclosure refers to the composition as disclosed herein for use as a medicament.

[0011e] In one aspect, the present disclosure refers to use of the composition as disclosed herein in the manufacture of a medicament for prevention of disease caused by the virus.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] **FIG. 1** shows a schematic of the mechanism for induction of cellular (CD4+ and CD8+ T cell) immune responses after intradermal injection of a temperature-controllable, self-replicating RNA (referred to herein as srRNAts and c-srRNA) vaccine.

[0013] **FIG. 2** shows a schematic diagram of SARS-CoV-2 nucleocapsid (N) proteins expressed from mRNA, self-replicating RNA, or temperature-sensitive, self-replicating RNA (srRNAts) delivered to mammalian host cells. In exemplary embodiments, the coding region of the N protein is the gene of interest (GOI) inserted within the srRNAts. The amino acid sequence of the G5004 antigen is set forth as SEQ ID NO:5. The G5004 antigen is a SARS-CoV-2 N protein devoid of a signal peptide. The amino acid sequence of the G5005 antigen is set forth as SEQ ID NO:6. The G5005 antigen is a fusion protein comprising the signal peptide sequence from the human CD5 antigen (CD5-SP) set forth as SEQ ID NO:8, and a SARS-CoV-2