COMPOSITIONS AND METHODS OF USE THEREOF

BACKGROUND

[0001] Alphaviruses are a group of small positive-sense single-stranded RNA viruses that are responsible for many diseases in humans and other animals. See, e.g., "The Alphaviruses: Gene Expression, Replication and Evolution," *Microbiological Reviews,* Sept. 1994, p. 491-562; Jose et al., "A structural and functional perspective of alphavirus replication and assembly," *Future Micriobol.,* 2009, v.4:837-856. Because of their high replication efficiency and specificity, alphaviruses have proven to be useful in the engineering of self-replicating RNA vectors for the expression of heterologous proteins in mammalian cells. See, e.g., Frolov et al., "Alphavirus-based expression vectors: strategies and applications", *PNAS*, 1996, v. 93, pp. 11371-11377; Young Kim, et al., "Enhancement of protein expression by alphavirus replicons by designing self-replicating subgenomic RNAs", *PNAS*, 2014, v.11:29, pp. 10708-10713. Stability of these RNA-based expression systems can degrade overtime. Additionally, ease of storage is often hindered by the need to keep these expression systems at impractical low temperatures. Accordingly, there remains a need to develop pharmaceutical compositions that comprise these vector systems that are stable when stored.

SUMMARY

[0002] Disclosed herein are pharmaceutical compositions comprising a lipid nanoparticle (LNP)-encapsulated self-amplifying alphavirus-based expression system or comprising a viral based expression system, further comprising a buffer system and two or more stabilizing excipients. Additionally, the present disclosure includes methods of inducing an immune response in a subject by administering a pharmaceutical composition to the subject.

[0002a] In one aspect, provided herein is a pharmaceutical composition comprising an RNA-based expression system, further comprising a buffer, an amino acid, and a cryoprotectant.

[0002b] In another aspect, provided herein is a pharmaceutical composition comprising a samRNA-based expression system, and further comprising 70-80 mM arginine; 11-15 wt% Sucrose; and 6-10 mM Tris; wherein the pharmaceutical composition has a pH of 7.6-8.0.

[0002c] In another aspect, provided herein is a pharmaceutical composition comprising a samRNA-based expression system, and further comprising about 75 mM arginine; about 13 wt% Sucrose; and about 8 mM Tris; wherein the pharmaceutical composition has a pH of about 7.8.

[0002d] In another aspect, provided herein is a use of the composition as described herein in the manufacture of a medicament for inducing an immune response in a subject, wherein the composition is to be administered to the subject.

[0002e] In another aspect, provided herein is a method of preparing the pharmaceutical composition as described herein, comprising a step of lyophilization.

[0002f] In another aspect, provided herein is a composition prepared by the method as described herein.

[0002g] In another aspect, provided herein is the composition as described herein, for use in therapy.

BRIEF DESCRIPTION OF THE DRAWINGS

[0003] FIG. 1A shows 6 line graphs illustrating concentration profile of DP in various formulations as described in Example 2.

[0004] FIG. 1B shows 6 line graphs illustrating % encapsulation profile of DP in various formulations as described in Example 2.

[0005] FIG. 2A shows 6 line graphs illustrating size profile of DP in various formulations as described in Example 2.

poly-A) generally can be inserted in the cassette following the payload-based sequences and before the viral vector sequences. A common intron sequence can also be derived from SV-40 and is referred to as the SV-40 T intron sequence. A cassette can also contain such an intron, located between the promoter/enhancer sequence and the payload(s). Selection of these and other common vector elements are conventional [see, e.g., Sambrook et al, "Molecular Cloning. A Laboratory Manual.", 2d edit., Cold Spring Harbor Laboratory, New York (1989) and references cited therein] and many such sequences are available from commercial and industrial sources as well as from Genbank.

[0174] A cassette can have one or more payloads (e.g., one or more payload-encoding nucleic acid sequences). For example, a given cassette can include 1-10, 1-20, 1-30, 10-20, 15-25, 15-20, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more payloads. Payloads can be linked directly to one another. Payloads can also be linked to one another with linkers. Payloads can be in any orientation relative to one another including N to C or C to N.

[0175] As described elsewhere herein, a cassette can be located in the site of any selected deletion in a viral vector, such as the deleted structural proteins of a VEE backbone or the site of the E1 gene region deletion or E3 gene region deletion of a ChAd-based vector, among others which may be selected.

[0176] The multicistronic samRNA vectors can be described using the following formula to describe the ordered sequence of each element, from 5' to 3':

$$P_1\text{-}(L5_b\text{-}N_c\text{-}L3_d)_X\text{-}P_2\text{-}(L5_b\text{-}N_c\text{-}L3_d)_X\text{-}P_a\text{-}(L5_b\text{-}N_c\text{-}L3_d)_X\text{-}(G5_e\text{-}U_f)Y\text{-}G3_g$$

wherein P_1 comprises the SGP1 subgenomic promoter, P_2 comprises the SGP2 subgenomic promoter where for P_a a = 0 or 1 for additional cassettes, N comprises a payload-encoding nucleic acid sequences, where c = 1, L5 comprises the 5' linker sequence, where b = 0 or 1, L3 comprises the 3' linker sequence, where d = 0 or 1, G5 comprises one of the at least one nucleic acid sequences encoding a GPGPG amino acid linker (SEQ ID NO: 1), where e = 0 or 1, G3 comprises one of the at least one nucleic acid sequences encoding a GPGPG amino acid linker (SEQ ID NO: 1), where g = 0 or 1, U comprises one of the at least one MHC class II epitope-encoding nucleic acid sequence, where f = 1, X = 1 to 400, where for each X the corresponding N_c is a corresponding payload-encoding nucleic acid sequence, and Y = 0, 1, or 2, where for each Y the corresponding U_f is a universal MHC class II epitope-encoding nucleic acid sequence, optionally wherein the at least one universal sequence comprises at least one of Tetanus toxoid and PADRE.

[0177] A payload encoding sequence (e.g., cassette or one or more of the nucleic acid sequences encoding a payload in the cassette) can be described using the following formula to describe the ordered sequence of each element, from 5' to 3':

$$P_a\text{-}(L5_b\text{-}N_c\text{-}L3_d)_X\text{-}(G5_e\text{-}U_f)_Y\text{-}G3_g$$

wherein P comprises the second promoter nucleotide sequence, where a = 0 or 1, where c = 1, N comprises one of the payload-derived nucleic acid sequences described herein (e.g., any of the antigen-encoding nucleic acid sequences described herein), L5 comprises the 5' linker sequence, where b = 0 or 1, L3 comprises the 3' linker sequence, where d = 0 or 1, G5 comprises one of the at least one nucleic acid sequences encoding a GPGPG amino acid linker (SEQ ID NO: 1), where e = 0 or 1, G3 comprises one of the at least one nucleic acid sequences encoding a GPGPG amino acid linker (SEQ ID NO: 1), where g = 0 or 1, U comprises one of the at least one MHC class II epitope-encoding nucleic acid sequence, where f = 1, X = 1 to 400, where for each X the corresponding N_c is a payload-encoding nucleic acid sequence, and Y = 0, 1, or 2, where for each Y the corresponding U_f is a (1) universal MHC class II epitope-encoding nucleic acid sequence, optionally wherein the at least one universal sequence comprises at least one of Tetanus toxoid and PADRE, or (2) a MHC class II epitope-encoding nucleic acid sequence. In some aspects, for each X the corresponding N_c is a distinct payload-encoding nucleic acid sequence. In some aspects, for each Y the corresponding U_f is a distinct universal MHC class II epitope-encoding nucleic acid sequence or a distinct MHC class II antigen-encoding nucleic nucleic acid sequence. The above payload encoding sequence formula in some instances only describes the portion of a cassette encoding concatenated payload sequences, such as concatenated T cell epitopes. For example, as an illustrative non-limiting example, in cassettes encoding concatenated T cell epitopes and one or more full-length SARS-CoV-2 proteins, the above payload encoding sequence formula describes the concatenated T cell epitopes and separately the cassette encodes one or more full-length SARS-CoV-2 proteins that are linked optionally using a multicistronic system, such as 2A ribosome skipping sequence elements (*e.g.*, E2A, P2A, F2A, or T2A sequences) and/or an Internal Ribosome Entry Site (IRES) sequence elements.

[0178] In one example, elements present include where b = 1, d = 1, e = 1, g = 1, h = 1, X = 18, Y = 2, and the vector backbone comprises a ChAdV68 vector, a = 1, P is a CMV promoter, the at least one second poly(A) sequence is present, wherein the second poly(A) sequence is an exogenous poly(A) sequence to the vector backbone, and optionally wherein the exogenous poly(A) sequence comprises an SV40 poly(A) signal sequence or a BGH

encoding nucleic acid sequence, and each epitope-encoding nucleic acid sequences (inclusive of optional 5' linker sequence and/or the optional 3' linker sequences) encodes a peptide 25 amino acids in length, the iterations can be separated by 75 nucleotides, such as in antigen-encoding nucleic acid represented by E_A-E_B-E_A…, E_A is separated by 75 nucleotides. In an illustrative example, an antigen-encoding nucleic acid having the sequence VTNTEMFVTAPDNLGYMYEVQWPGQTQPQIANCSVYDFFVWLHYYSVRD TVTNTEMFVTAPDNLGYMYEVQWPGQTQPQIANCSVYDFFVWLHYYSVRDT (SEQ ID NO: 2) encoding iterations of 25mer antigens Trp1 (VTNTEMFVTAPDNLGYMYEVQWPGQ (SEQ ID NO: 3)) and Trp2 (TQPQIANCSVYDFFVWLHYYSVRDT (SEQ ID NO: 4)), the iterations of Trp1 are separated by the 25mer Trp2 and thus the iterations of the Trp1 epitope-encoding nucleic acid sequences are separated the 75 nucleotide Trp2 epitope-encoding nucleic acid sequence. In examples where iterations are separated by 2, 3, 4, 5, 6, 7, 8, or 9 separate distinct epitope-encoding nucleic acid sequence, and each epitope-encoding nucleic acid sequences (inclusive of optional 5' linker sequence and/or the optional 3' linker sequences) encodes a peptide 25 amino acids in length, the iterations can be separated by 150, 225, 300, 375, 450, 525, 600, or 675 nucleotides, respectively.

[0233] In one embodiment, different peptides and/or polypeptides or nucleotide sequences encoding them are selected so that the peptides and/or polypeptides capable of associating with different MHC molecules, such as different MHC class I molecules and/or different MHC class II molecules. In some aspects, one vaccine composition comprises coding sequence for peptides and/or polypeptides capable of associating with the most frequently occurring MHC class I molecules and/or different MHC class II molecules. Hence, vaccine compositions can comprise different fragments capable of associating with at least 2 preferred, at least 3 preferred, or at least 4 preferred MHC class I molecules and/or different MHC class II molecules.

[0234] The vaccine composition can be capable of stimulating a specific cytotoxic T-cell response and/or a specific helper T-cell response. The vaccine composition can be capable of stimulating a specific cytotoxic T-cell response and a specific helper T-cell response.

[0235] The vaccine composition can be capable of stimulating a specific B-cell response (*e.g.*, an antibody response).

[0236] The vaccine composition can be capable of stimulating a specific cytotoxic T-cell response, a specific helper T-cell response, and/or a specific B-cell response. The vaccine composition can be capable of stimulating a specific cytotoxic T-cell response and a

subgenomic RNA provides for expression of the heterologous protein. Traditionally, additional vectors that expresses the structural proteins are then supplied *in trans,* such as by co-transfection of a cell line, to produce infectious virus. A system is described in detail in USPN 8,093,021, which is herein incorporated by reference in its entirety, for all purposes. The helper vector system provides the benefit of limiting the possibility of forming infectious particles and, therefore, improves biosafety. In addition, the helper vector system reduces the total vector length, potentially improving the replication and expression efficiency. Thus, an example of an antigen expression vector described herein can utilize an alphavirus backbone wherein the structural proteins are replaced by an antigen cassette, the resulting vector both reducing biosafety concerns, while at the same time promoting efficient expression due to the reduction in overall expression vector size.

Self-Amplifying Virus Production *in vitro*

[0264] A convenient technique well-known in the art for RNA production is *in vitro* transcription(IVT). In this technique, a DNA template of the desired vector is first produced by techniques well-known to those in the art, including standard molecular biology techniques such as cloning, restriction digestion, ligation, gene synthesis (*e.g.*, chemical and/or enzymatic synthesis), and polymerase chain reaction (PCR).

[0265] The DNA template contains an RNA polymerase promoter at the 5' end of the sequence desired to be transcribed into RNA (*e.g.*, SAM). Promoters include, but are not limited to, bacteriophage polymerase promoters such as T3, T7, K11, or SP6. Depending on the specific RNA polymerase promoter sequence chosen, additional 5' nucleotides can be transcribed in addition to the desired sequence. For example, the canonical T7 promoter can be referred to by the sequence TAATACGACTCACTATAGG (SEQ ID NO: 5), in which an IVT reaction using the DNA template TAATACGACTCACTATAGGN (SEQ ID NO: 6) for the production of desired sequence N will result in the mRNA sequence GG-N. In general, and without wishing to be bound by theory, T7 polymerase more efficiently transcribes RNA transcripts beginning with guanosine. In instances where additional 5' nucleotides are not desired (*e.g.*, no additional GG), the RNA polymerase promoter contained in the DNA template can be a sequence the results in transcripts containing only the 5' nucleotides of the desired sequence, *e.g.*, a samRNA having the native 5' sequence of the self-replicating virus from which the samRNA vector is derived. For example, a minimal T7 promoter can be referred to by the sequence TAATACGACTCACTATA (SEQ ID NO: 7), in which an IVT reaction using the DNA template TAATACGACTCACTATAN (SEQ ID NO: 8) for the production of desired sequence N will result

in the mRNA sequence N. Likewise, a minimal SP6 promoter referred to by the sequence ATTTAGGTGACACTATA (SEQ ID NO: 9) can be used to generate transcripts without additional 5' nucleotides. In a typical IVT reaction, the DNA template is incubated with the appropriate RNA polymerase enzyme, buffer agents, and nucleotides (NTPs).

[0266] The resulting RNA polynucleotide can optionally be further modified including, but limited to, addition of a 5' cap structure such as 7-methyl guanosine or a related structure, and optionally modifying the 3' end to include a polyadenylate (polyA) tail. In a modified IVT reaction, RNA is capped with a 5' cap structure co-transcriptionally through the addition of cap analogues during IVT. Cap analogues can include dinucleotide (m^7G-ppp-N) cap analogues or trinucleotide (m^7G-ppp-N-N) cap analogues, where N represents a nucleotide or modified nucleotide (*e.g.*, ribonucleosides including, but not limited to, adenosine, guanosine, cytidine, and uridine). Exemplary cap analogues and their use in IVT reactions are also described in greater detail in U.S. Pat. No. 10,519,189, herein incorporated by reference for all purposes. As discussed, T7 polymerase more efficiently transcribes RNA transcripts beginning with guanosine. To improve transcription efficiency in templates that do not begin with guanosine, a trinucleotide cap analogue (m^7G-ppp-N-N) can be used. The trinucleotide cap analogue can increase transcription efficiency 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20-fold or more relative to an IVT reaction using a dinucleotide cap analogue (m^7G-ppp-N).

[0267] A 5' cap structure can also be added following transcription, such as using a vaccinia capping system (*e.g.*, NEB Cat. No. M2080) containing mRNA 2'-O-methyltransferase and S-Adenosyl methionine.

[0268] The resulting RNA polynucleotide can optionally be further modified separately from or in addition to the capping techniques described including, but limited to, modifying the 3' end to include a polyadenylate (polyA) tail.

[0269] The RNA can then be purified using techniques well-known in the field, such as phenol-chloroform extraction or column purification (*e.g.*, chromatography-based purification).

Delivery via lipid nanoparticle

[0270] An important aspect to consider in vaccine vector design is immunity against the vector itself (Riley 2017). This may be in the form of preexisting immunity to the vector itself, such as with certain human adenovirus systems, or in the form of developing