

RECOMBINANT ROTAVIRUS EXPRESSING EXOGENOUS PROTEIN AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of U.S. Provisional Application No. 63/256,960, filed October 18, 2021, and U.S. Provisional Application No. 63/256,875, filed October 18, 2021, each of which is incorporated by reference herein in its entirety.

STATEMENT OF GOVERNMENTAL RIGHTS

[0002] This invention was made with government support under TR002529 and AI144881 awarded by the National Institutes of Health. The Government has certain rights in the invention.

SEQUENCE LISTING

[0003] A Sequence Listing accompanies this application and is submitted as an .xml file of the sequence listing in ST26 format named “144578_00351.xml” which is 120,469 bytes in size and was created on October 18, 2022. The sequence listing is electronically submitted via EFS-Web with the application and is incorporated herein by reference in its entirety.

BACKGROUND

[0004] Rotavirus (RV) and norovirus (NoV) are the leading cause of acute gastroenteritis (AGE) and acute diarrheal episodes in young children and the elderly (1, 2). The development and introduction of effective RV vaccines- a monovalent vaccine (RV1), Rotarix (GSK Biologicals) and a pentavalent vaccine (RV5), RotaTeq (Merck and Company), in the childhood immunization programs in the US and many other countries resulted in reducing the incidence of RV hospitalizations and mortality, and these vaccines are quite successful in generating neutralizing antibodies in vaccinated children (3, 4). In countries where RV vaccines are largely used, an increase of NoV mediated diarrheal diseases and hospitalizations in children during the first 5 years of life became more evident (5, 6). The incidence of NoV disease is higher in young children, in the elderly population, and in subjects with lower immune defenses, for whom effective preventive measures are needed urgently (7). NoV disease is extremely contagious, only 10

comprises a linker. In some embodiments, the linker is a GAG flexible linker or a GSG flexible linker. In some embodiments, the heterologous polynucleotide is about 2.6 kb or less in length. In some embodiments, the heterologous polynucleotide is about 1.55 kb or less in length. In some embodiments, the heterologous polynucleotide is about 1.3 kb or less in length. In some embodiments, the peptide or protein is a glycoprotein. In some embodiments, the peptide or protein comprises one or more glycosylation site. In some embodiments, the heterologous polynucleotide encodes a reporter. In some embodiments, the reporter is selected from a fluorescent reporter, an enzyme, and an antigen tag. In some embodiments, the reporter is fused in-frame 3' to the sequence encoding the RV protein. In some embodiments, the polynucleotides encoding a protein selected from SEQ ID NOs: 2-11, or a sequence with at least about 90% identity to a sequence selected from SEQ ID NOs: 2-11. In some embodiments, the polynucleotides comprise any one of SEQ ID NOs: 13-22 or a sequence with at least about 90% identity to any one of SEQ ID NOs: 13-22.

[0005a] In one aspect, provided herein is a polynucleotide comprising:

a sequence encoding a rotavirus (RV) NSP3 protein; and

a heterologous polynucleotide, wherein the heterologous polynucleotide encodes a peptide or protein and is in frame with the NSP3, and wherein the peptide or protein comprises a viral peptide or protein.

[0005b] In another aspect, provided herein is an infectious particle comprising the polynucleotide described herein.

[0005c] In another aspect, provided herein is a pharmaceutical composition comprising the infectious particle described herein, optionally, further comprising a pharmaceutically acceptable carrier or excipient.

[0005d] In another aspect, provided herein is the use of the pharmaceutical composition described herein in the manufacture of a medicament for eliciting an immune response to one or more microorganism in a subject, wherein an effective amount of the pharmaceutical composition is to be administered to the subject to elicit an immune response to the one or more microorganism.

[0005e] In another aspect, provided herein is the use of the pharmaceutical composition described herein in the manufacture of a medicament for vaccinating a subject against one or more pathogens, wherein an effective amount of the pharmaceutical composition is to be administered to the subject to vaccinate said subject against the one or more pathogens.

[0005f] In another aspect, provided herein is a method of generating recombinant rotavirus (RV) *in vitro* comprising:

introducing a polynucleotide described herein into a cell; allowing the cell to express the polynucleotide; incubating the cells for a sufficient time to produce RV; and harvesting virus produced by the cells to generate RV *in vitro*.

[0005g] In another aspect, provided herein is a system, platform, or kit for generating recombinant rotavirus (RV) comprising:

- (a) a polynucleotide described herein; and
- (b) cells capable of expressing the polynucleotides of (a).

[0005h] In another aspect, provided herein is the pharmaceutical composition described herein for use in therapy.

[0006] In another aspect of the current disclosure, infectious particles are provided. In some embodiments, the infectious particles comprise a polynucleotide comprising a sequence encoding a rotavirus (RV) NSP3 protein; and a heterologous polynucleotide. In some embodiments, the polynucleotide is operably linked to a promoter. In some embodiments, the promoter is a T7 promoter or a T3 promoter. In some embodiments, the promoter is a T7 promoter. In some embodiments, the polynucleotide encodes a positive sense viral transcript. In some embodiments, the sequence encoding an RV NSP3 is a sequence encoding SEQ ID NO: 1, or a sequence with at least about 90% identity to SEQ ID NO: 1. In some embodiments, the heterologous polynucleotide encodes a peptide or protein. In some embodiments, the heterologous polynucleotide encodes a peptide or protein and is in frame with the NSP3. In some embodiments, the peptide or protein comprises an antigenic peptide or protein. In some embodiments, the peptide or protein comprises a microorganismal peptide or protein. In some embodiments, the peptide or protein comprises a bacterial peptide or protein. In some embodiments, the peptide or protein comprises a viral peptide or protein. In some embodiments, the peptide or protein comprises a norovirus (NoV) peptide or protein. In some embodiments, the NoV peptide or protein comprises NoV VP1 protein. In some embodiments, the NoV peptide or protein is selected from SEQ ID NOs: 24, 26, or 80-84. In some embodiments, the peptide or protein comprises a SARS-CoV-2 protein or peptide. In some embodiments, the SARS-CoV-2 protein or peptide is selected from an N protein, an S protein, or fragment of either an N protein or an S protein. In some embodiments, the SARS-CoV-2 protein