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VACCINES AGAINST HEPATITIS B VIRUS

Description

1. INTRODUCTION

[0001] Provided herein are genetically modified arenaviruses suitable as vaccines for prevention and treatment of Hepatitis B virus infections. Also provided herein are pharmaceutical compositions and pharmaceutical compositions for use in methods for the treatment of Hepatitis B virus infections. Specifically, provided herein are pharmaceutical compositions, vaccines, and pharmaceutical compositions and vaccines for use in methods of treating or preventing Hepatitis B virus infections. As such, the present application provides immunotherapies for Hepatitis B virus infections.

2. BACKGROUND

2.1 The pathogen and the disease

[0002] Hepatitis B virus (HBV) is a double-stranded enveloped virus of the Hepadnaviridae family. The virus particle consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein. The nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity. The outer envelope contains embedded proteins which are involved in viral binding of, and entry into, susceptible cells. HBV replicates in the hepatocytes of humans and other higher primates, but does not grow in artificial cell cultures.

[0003] The outcomes of HBV infection are age-dependent and include asymptomatic infection, acute hepatitis B, chronic HBV infection, cirrhosis and hepatocellular carcinoma (HCC). Acute hepatitis B occurs in approximately 1% of perinatal infections, 10% of early childhood infections (children aged 1-5 years) and 30% of late infections (people aged >5 years). Fulminant hepatitis develops in 0.1-0.6% of acute hepatitis cases; mortality from fulminant hepatitis B is approximately 70%. The development of chronic HBV infection is inversely related to the age of acquisition, occurring in approximately 80-90% of people infected perinatally, about 30% of children infected before the age of 6 years, and in <5% of infections occurring in otherwise healthy adults (Hyams et al., 1995, Clinical Infections Diseases 20:992-1000). Comorbidities, including concurrent HIV infection and ingestion

of alcohol or aflotoxins, or both, may have an important role in the development of morbidity related to hepatitis B. It is estimated that 10% of the 40 million people infected with HIV worldwide are coinfected with HBV.

[0004] People with chronic HBV infection have a 15-25% risk of dying prematurely from HBV-related cirrhosis and HCC (Beasley and Hwang, 1991, Proceedings of the 1990 International Symposium on Viral Hepatitis and Liver Disease: Contemporary Issues and Future Prospects 532-535). Acute HBV infection is characterized by the presence of HBsAg, the surface antigen of HBV, and immunoglobulin M (IgM) antibody to the core antigen, HBcAg. During the initial, highly replicative phase of infection, patients are also seropositive for HBeAg, the extracellular and secreted form of HBcAg which can be found in the serum of patients where it serves as a marker of active replication in chronic hepatitis. Antibody to HBsAg (anti-HBs) is discernible after a few weeks and is followed by clearance of the HBsAg. Chronic infection is characterized by the persistence (>6 months) of HBsAg (with or without concurrent HBeAg). Persistence of HBsAg is the principal marker of risk for developing chronic liver disease and HCC later in life. The presence of HBeAg indicates that the blood and body fluids of the infected individual are highly contagious.

2.2 Epidemiology and public health

[0005] Diseases caused by the hepatitis B virus have a worldwide distribution. It is estimated that two billion people have at some time been infected with HBV. Of these, approximately 360 million individuals are chronically infected and at risk of serious illness and death, mainly from liver cirrhosis and hepatocellular carcinoma (HCC). Mathematical modeling for the year 2000 estimated the number of deaths from HBV-related diseases at about 600 000 each year worldwide (Goldstein et al., 2005, International J. Epidemiology 34:1329-1339). Humans are the only reservoir of HBV. The virus is transmitted by percutaneous and permucosal exposure to infected blood and other body fluids, mainly semen and vaginal fluid. The incubation period is 75 days on average, but may vary from about 30 days to 180 days. The surface antigen of HBV (HBsAg) may be detected in serum 30-60 days following infection and may persist for widely variable periods of time. The endemicity of hepatitis B is described by the prevalence of HBsAg in the general population of a defined geographical area, and it varies considerably globally: HBsAg prevalences of >8% are typical of highly endemic areas, prevalences of 2-7% are

found in areas of intermediate endemicity, whereas in areas with low endemicity <2% of the population is HBsAg-positive.

[0006] In highly endemic areas, HBV is most commonly spread from mother to child at birth, or from person to person in early childhood (Goldstein et al., 2005, International J. Epidemiology 34:1329-1339; Wong et al., 1984, Lancet 1:921-926; de la Hoz et al., 2008 International J. Infectious Diseases 12:183-189). Perinatal or early childhood transmission may also account for more than one third of chronic infections in areas of low endemicity (Margolis et al., 1995, JAMA 274:1201-1208) although in those settings, sexual transmission and the use of contaminated needles, especially among injecting drug users, are the major routes of infection (Goldstein et al., 2002, J. Infectious Diseases 185:713-719).

2.3 Current treatment

[0007] Universal hepatitis B vaccination has been shown to reduce the rates of HBV infection and HCC significantly. However, once chronic HBV infection is established, treatment still poses a major challenge as traditional therapies usually fail to provide sustained control of viral replication and liver damage in most patients.

[0008] Currently approved antiviral treatments for chronic hepatitis B include pegylated (PEG) recombinant interferon-a and viral DNA polymerase inhibitors. These agents decrease viral replication and have been shown to delay progression of cirrhosis, reduce the incidence of HCC and improve long-term survival. However, treatment is complicated by the toxicity of the agents and it can only cure a small subset of chronically infected individuals. Although viral levels in the blood plummet to almost undetectable levels in individuals receiving standard therapies, reductions of intrahepatic viral DNA are only modest. As a consequence, rebound of viraemia frequently occurs after discontinuation of treatment and people with chronic HBV infections must stay on lifelong treatment. However, even after ten years on antiviral therapy, drugs reduce liver failure by only 40-70%, and mortality from cirrhosis and liver cancer remains high.

2.4 Hepatitis B and the immune system

[0009] Chronic hepatitis B infection is characterized by dysfunctional innate and adaptive antiviral immunity (Bertoletti & Ferrari, 2012, Gut 61:1754-1764). In contrast, HBV-specific immunity in patients with resolved HBV infection is robust and multifunctional.

Several mechanisms might contribute to the dysfunction of HBV-specific T-cell immunity in chronic hepatitis B patients, including high levels of viral antigenaemia, and the tolerizing microenvironment of the liver (Jenne & Kubes, 2013, Nat. Immunol. 14:996-1006). Previous studies have demonstrated that suppression of viral replication can transiently and partially restore antiviral T-cell immunity, which supports the hypothesis that long-term exposure to high levels of antigenaemia might cause dysfunction of antiviral T cells (Boni et al., 2003, J. Hepatol. 39:595-605).

[0010] Therapeutic vaccines that could reverse the dysfunctional immune state of chronic hepatitis B patients and restore antiviral immunity, would theoretically have the potential to eliminate viremia and reduce intrahepatic levels of HBV DNA to zero, thus holding great promise for HBV cure.

[0011] Recently, HBV vaccines have been identified as a promising therapeutic strategy for treatment and control of HBV infection in HBV carriers and persistently infected patients (Michel & Tiollais, 2010, Pathol. Biol. (Paris) 58:288-295; Liu et al., 2014, Virol. Sin. 29:10-16). In about 50% of chronic active HBV patients specific therapy by conventional anti-HBV vaccination effectively reduced the replication of HBV and inhibited the immune tolerance to HBsAg protein (Couillin et al., 1999, J. Infect. Dis. 180:15-26). However, so far monotherapy with HBsAg based vaccines did not lead to sustained control of HBV replication and/or liver damage (Akbar et al., 2013, Hepatobiliary Pancreat. Dis. Int. 12:363-369) and new therapy strategies are needed to provide potent and durable antiviral immune responses and long-term control of HBV replication.

[0012] The failure of previous therapeutic vaccine approaches highlights the challenges and limitations of current knowledge regarding immune responses in chronic HBV infection (Michel et al., 2011, J. Hepatol. 54:1286-1296). The combination of a high viral load condition such as chronic hepatitis B with the tolerizing liver microenvironment might make it difficult to achieve full recovery of antiviral T-cell immunity.

[0013] Intensive research is currently concentrated on a better understanding of immune responses in hepatocytes, on mechanisms by which HBV evades innate immunity and on proper selection of patients susceptible to benefit from immune therapy, which could increase the efficacy of therapeutic vaccination (Michel et al., 2015, Med. Microbiol. Immunol. 204:121-129).

[0014] In the first aspect, the invention provides an infectious arenavirus viral vector, wherein an arenavirus open reading frame is removed and replaced by a nucleotide sequence selected from the group consisting of:

a. a nucleotide sequence encoding an HBV HBc protein or an antigenic fragment thereof;
b. a nucleotide sequence encoding a fusion of HBV HBs and HBc proteins or antigenic fragments thereof; and
c. a nucleotide sequence encoding an HBV pre-S2/S protein or an antigenic fragment thereof;

wherein the viral vector is capable of eliciting a T cell response against the HBV HBc protein, the fusion of HBV HBs and HBc proteins, the HBV pre-S2/S protein, or an antigenic fragment thereof.

[0015] In the second aspect, the invention provides a pharmaceutical composition, immunogenic composition, or vaccine comprising the viral vector of the invention and a pharmaceutically acceptable carrier.

[0016] In the third aspect, the invention provides a viral vector, a pharmaceutical composition, immunogenic composition, or vaccine of the invention, for use in a method of treating or preventing a Hepatitis B virus infection in a patient.

[0017] In the fourth aspect, the invention provides an isolated nucleic acid, wherein the nucleic acid comprises an arenavirus genomic segment wherein one open reading frame of the genomic segment is deleted or functionally inactivated and wherein the genomic segment comprises one or more of:

a. a nucleotide sequence encoding an HBV HBc protein or an antigenic fragment thereof;
b. a nucleotide sequence encoding a fusion of HBV HBs and HBc proteins or antigenic fragments thereof; or
c. a nucleotide sequence encoding an HBV pre-S2/S protein or an antigenic fragment thereof;

wherein a viral vector comprising the arenavirus genomic segment is capable of eliciting a T cell response against the HBV HBc protein, the fusion of HBV HBs and HBc proteins, the HBV pre-S2/S protein, or an antigenic fragment thereof; optionally wherein the genomic segment is the short segment, wherein the open reading frame encoding the glycoprotein (GP) is deleted.

[0018] In the fifth aspect, the invention provides a cDNA of the arenavirus genomic segment as defined in the fourth aspect.

[0019] In the sixth aspect, the invention provides an *in vitro* method for generating an infectious, replication-deficient arenavirus viral vector comprising:

a. transfecting into a host cell the cDNA of the invention;

b. maintaining the host cell under conditions suitable for virus formation; and

c. harvesting the infectious, replication-deficient arenavirus viral vector;

wherein the host cell expresses the open reading frame of the genomic segment that is deleted or functionally inactivated ;

optionally wherein the method further comprises in step a. transfecting into the host cell: a cDNA of a second arenavirus genomic segment, a nucleic acid comprising the L protein ORF, and/or a nucleic acid comprising the NP ORF.

[0020] In the seventh aspect, the invention provides a pharmaceutical composition comprising a first infectious, replication-deficient arenavirus viral vector engineered to contain a genome with the ability to amplify and express its genetic information in infected cells but unable to produce further infectious progeny particles in normal, not genetically engineered cells, wherein one arenavirus open reading frame is removed and replaced by a first nucleotide sequence selected from the group consisting of:

a. a nucleotide sequence encoding an HBV HBc protein or an antigenic fragment thereof;

b. a nucleotide sequence encoding a fusion of HBV HBs and HBc proteins or antigenic fragments thereof; and

c. a nucleotide sequence encoding an HBV pre-S2/S protein or an antigenic fragment thereof;

and a second infectious, replication-deficient arenavirus viral vector engineered to contain a genome with the ability to amplify and express its genetic information in infected cells but unable to produce further infectious progeny particles in normal, not genetically engineered cells, wherein one arenavirus open reading frame is removed and replaced by a second nucleotide sequence selected from the group consisting of:

a. a nucleotide sequence encoding an HBV HBc protein or an antigenic fragment thereof;

b. a nucleotide sequence encoding a fusion of HBV HBs and HBc proteins or antigenic fragments thereof; and

c. a nucleotide sequence encoding an HBV pre-S2/S protein or an antigenic fragment thereof;

wherein the first and second nucleotide sequences are different; wherein the first viral vector and the second viral vector are capable of eliciting a T cell response against the HBV HBc protein, the fusion of HBV HBs and HBc proteins, the HBV pre-S2/S protein, or an antigenic fragment thereof.

[0021] Hereinafter, reference to the arenavirus in the context of the invention, or the arenavirus comprised in the pharmaceutical composition of the invention, means the arenavirus viral vector.

3. SUMMARY OF THE INVENTION

[0022] The present application provides immunotherapies for Hepatitis B virus infections. Provided herein is an infectious arenavirus viral vector, wherein an arenavirus open reading frame is removed and replaced by a nucleotide sequence selected from the group consisting of:

a. a nucleotide sequence encoding an HBV pre-S2/S protein or an antigenic fragment thereof;
b. a nucleotide sequence encoding an HBV HBc protein or an antigenic fragment thereof; and
c. a nucleotide sequence encoding a fusion of HBV HBs and HBc proteins or antigenic fragments thereof; and

wherein the viral vector is capable of eliciting a T cell response against the HBV HBc protein, the fusion of HBV HBs and HBc proteins, the HBV pre-S2/S protein, or an antigenic fragment thereof.

[0023] In certain embodiments, the infectious arenavirus viral vector is replication-deficient (See Section 6.1(a)). In certain embodiments, the infectious arenavirus viral vector is replication-competent (See Section 6.1(b)). In certain embodiments, the infectious, replication-deficient arenavirus viral vector is bisegmented. In certain embodiments, the infectious, replication-deficient arenavirus viral vector is trisegmented. In certain embodiments, the infectious, replication-competent arenavirus viral vector is trisegmented.

[0024] In certain embodiments, provided herein is an arenavirus viral vector, wherein an arenavirus open reading frame is removed and replaced by a nucleotide sequence selected from the group consisting of:

a. a nucleotide sequence encoding an HBV pre-S2/S protein or an antigenic fragment thereof;
b. a nucleotide sequence encoding an HBV HBc protein or an antigenic fragment thereof; and
c. a nucleotide sequence encoding a fusion of HBV HBs and HBc proteins or antigenic fragments thereof; and wherein the viral vector is capable of eliciting a T

cell response against the HBV HBc protein, the fusion of HBV HBs and HBc proteins, the HBV pre-S2/S protein, or an antigenic fragment thereof.

[0025] In certain embodiments, the arenavirus viral vector is replication-deficient. In certain embodiments, the arenavirus viral vector is replication-competent.

[0026] In certain embodiments, a viral vector as provided herein is infectious, i.e., is capable of entering into or injecting its genetic material into a host cell. In certain more specific embodiments, a viral vector as provided herein is infectious, i.e., is capable of entering into or injecting its genetic material into a host cell followed by amplification and expression of its genetic information inside the host cell. In certain embodiments, the viral vector is an infectious, replication-deficient arenavirus viral vector engineered to contain a genome with the ability to amplify and express its genetic information in infected cells but unable to produce further infectious progeny particles in normal, not genetically engineered cells. In certain embodiments, provided herein is a cell line that supports viral growth of a wild type virus but does not express the complementing viral protein, thus is unable to produce further infectious viral progeny particles. In certain embodiments, the infectious arenavirus viral vector is replication-competent and able to produce further infectious progeny particles in normal, not genetically engineered cells.

[0027] In certain embodiments, the pre-S2/S protein or the antigenic fragment thereof comprises an amino acid sequence that is 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 1. In certain embodiments, the fragment is antigenic when it is capable of (i) eliciting an antibody immune response in a host (e.g., mouse, rabbit, goat, or donkey) wherein the resulting antibodies bind specifically to human HBV pre-S2/S protein; and/or (ii) eliciting a specific T cell immune response.

[0028] In certain embodiments, the HBc protein or the antigenic fragment thereof comprises an amino acid sequence that is 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 2. In certain embodiments, the fragment is antigenic when it is capable of (i) eliciting an antibody immune response in a host (e.g., mouse, rabbit, goat, or donkey) wherein the resulting antibodies bind specifically to human HBV HBc protein; and/or (ii) eliciting a specific T cell immune response.

[0029] In certain embodiments, the fusion of HBV HBs and HBc proteins or antigenic fragments thereof comprises an amino acid sequence that is 80%, 81%, 82%, 83%, 84%,

85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 3. In certain embodiments, the fragment is antigenic when it is capable of (i) eliciting an antibody immune response in a host (e.g., mouse, rabbit, goat, or donkey) wherein the resulting antibodies bind specifically to human HBV HBs, HBc or both HBs and HBc; and/or (ii) eliciting a specific T cell immune response.

[0030] The HBe protein or the antigenic fragment thereof comprises an amino acid sequence that is 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 26. The fragment may be antigenic when it is capable of (i) eliciting an antibody immune response in a host (e.g., mouse, rabbit, goat, or donkey) wherein the resulting antibodies bind specifically to human HBV HBe protein; and/or (ii) eliciting a specific T cell immune response.

[0031] In certain embodiments, the viral vector comprises at least two of:

a. a nucleotide sequence encoding an HBV pre-S2/S protein or an antigenic fragment thereof;
b. a nucleotide sequence encoding an HBV HBc protein or an antigenic fragment thereof; and
c. a nucleotide sequence encoding a fusion of HBV HBs and HBc proteins or antigenic fragments thereof; and

wherein the viral vector is capable of eliciting a T cell response against the HBV HBc protein, the fusion of HBV HBs and HBc proteins, the HBV pre-S2/S protein, or an antigenic fragment thereof.

[0032] In certain embodiments, the viral vector comprises at least three of:

a. a nucleotide sequence encoding an HBV pre-S2/S protein or an antigenic fragment thereof;
b. a nucleotide sequence encoding an HBV HBc protein or an antigenic fragment thereof; and
c. a nucleotide sequence encoding a fusion of HBV HBs and HBc proteins or antigenic fragments thereof; and

wherein the viral vector is capable of eliciting a T cell response against the HBV HBc protein, the fusion of HBV HBs and HBc proteins the HBV pre-S2/S protein, or an antigenic fragment thereof.

[0033] In certain embodiments, an open reading frame (ORF) of the arenavirus is deleted or functionally inactivated and replaced with a nucleic acid encoding an HBV antigen as

described herein. In a specific embodiment, the ORF that encodes the glycoprotein GP of the arenavirus is deleted or functionally inactivated. In certain embodiments, functional inactivation of a gene eliminates any translation product. In certain embodiments, functional inactivation refers to a genetic alteration that allows some translation, the translation product, however, is not longer functional and cannot replace the wild type protein.

[0034] In certain embodiments, the viral vector can amplify and express its genetic information in a cell that has been infected by the viral vector but the viral vector is unable to produce further infectious progeny particles in a non-complementing cell. In certain embodiments, a viral vector as provided herein is infectious, i.e., is capable of entering into or injecting its genetic material into a host cell. In certain more specific embodiments, a viral vector as provided herein is infectious, i.e., is capable of entering into or injecting its genetic material into a host cell followed by amplification and expression of its genetic information inside the host cell.

[0035] In certain embodiments, the genomic information encoding the infectious arenavirus particle is derived from the lymphocytic choriomeningitis virus (LCMV) Clone 13 strain or the LCMV MP strain. The nucleotide sequence of the S segment and of the L segment of Clone 13 are set forth in SEQ ID NOs: 12 and 7, respectively.

[0036] In certain embodiments, provided herein is a viral vector whose genome is or has been derived from the genome of Clone 13 (SEQ ID NOs: 12 and 7) by deleting an ORF of the Clone 13 genome (e.g., the ORF of the GP protein) and replacing it with a heterologous ORF that encodes an antigen (e.g., an HBV antigen) such that the remaining LCMV genome is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, at least 99%, or 100% identical to the nucleotide sequence of Clone 13 (SEQ ID NOs: 12 and 7).

[0037] In certain embodiments, provided herein is a viral vector whose genome has been derived from the genome of the LCMV strain MP (SEQ ID NOs: 13 and 14) by deleting an ORF of the LCMV strain MP genome (e.g., the ORF of the GP protein) and replacing it with a heterologous ORF that encodes an antigen (e.g., an HBV antigen) such that the remaining LCMV genome is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, at least 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, at least 99.9% or 100% identical to the nucleotide sequence of LCMV strain MP (SEQ ID NOs: 13 and 14).

[0038] In a more specific embodiment, the viral vector comprises a genomic segment, wherein the genomic segment comprises a nucleotide sequence that is at least 90%,

91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, at least 99%, or 100% identical to the sequence of nucleotide 1639 to 3315 of SEQ ID NO: 11 or 1640 to 3316 of SEQ ID NO: 12. In certain embodiments, the viral vector comprises a genomic segment comprising a nucleotide sequence encoding an expression product whose amino acid sequence is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, at least 99%, or 100% identical to the amino acid sequence encoded by 1639 to 3315 of SEQ ID NO: 11 or 1640 to 3316 of SEQ ID NO: 12.

[0039] Also provided herein are isolated nucleic acids, wherein the nucleic acid is a cDNA of an arenavirus genomic segment wherein one ORF of the genomic segment is deleted or functionally inactivated and wherein the genomic segment comprises one or any combination of:

a. a nucleotide sequence encoding an HBV pre-S2/S protein or an antigenic fragment thereof;
b. a nucleotide sequence encoding an HBV HBc protein or an antigenic fragment thereof; and
c. a nucleotide sequence encoding a fusion of HBV HBs and HBc proteins or antigenic fragments thereof; and

wherein a viral vector comprising the arenavirus genomic segment is capable of eliciting a T cell response against the HBV pre-S2/S protein, the HBV HBc protein, or the fusion of HBV HBs and HBc proteins, or an antigenic fragment thereof.

[0040] In certain embodiments, the genomic segment is the short segment, wherein the ORF encoding the GP is deleted.

[0041] In one aspect, provided herein are methods for generating an infectious, replication-deficient arenavirus particle comprising:

a. transfecting into a host cell a nucleic acid described herein;
b. maintaining the host cell under conditions suitable for virus formation; and
c. harvesting the infectious, replication-deficient arenavirus particle;

wherein the host cell expresses the ORF that is deleted or functionally inactivated on the genomic segment. In certain embodiments, any additional nucleic acids required for the rescue of a viral particle are also transfected into the host cell in step a. Such additional nucleic acids can be: the cDNA of the second arenavirus genomic segment, a nucleic acid comprising the L protein ORF, and/or a nucleic acid comprising the NP ORF.

[0042] In another aspect, provided herein are compositions, e.g., pharmaceutical, immunogenic or vaccine compositions, comprising a viral vector described herein and a pharmaceutically acceptable carrier. Also provided herein are compositions (e.g., vaccine

compositions) that comprise two or more different viral vectors described herein (i.e., wherein the viral vectors encode different HBV antigens). In certain embodiments, the pharmaceutical composition comprises a nucleic acid or fusion protein described herein.

[0043] In a further aspect, methods of treating or preventing HBV infection in a patient, comprise administering to the patient a viral vector, a pharmaceutical composition, an immunogenic composition, or a vaccine described herein. In yet another aspect, provided herein is use of a viral vector, a pharmaceutical composition, an immunogenic composition, or a vaccine described herein in methods of treatment or prevention of HBV. In certain embodiments, an infectious arenavirus viral vector expressing an HBV antigen or a fragment thereof is capable of preventing transmission and/or infection of HBV from a mother to an unborn child. In certain embodiments, one or more infectious arenavirus viral vectors expressing an HBV antigen or a fragment thereof are capable of preventing transmission and/or infection of HBV from a mother to an unborn child. In certain embodiments, the infectious arenavirus viral vector is replication-deficient (See Section 6.1(a)). In certain embodiments, the infectious arenavirus viral vector is replication-competent (See Section 6.1(b)).

[0044] In certain embodiments, administering to a patient an infectious arena virus viral vector expressing an HBV antigen or a fragment thereof induces a long-lasting immune response. In certain embodiments, the infectious arenavirus viral vector is replication-deficient (See Section 6.1(a)). In certain embodiments, the infectious arenavirus viral vector is replication-competent (See Section 6.1(b)).

[0045] In certain embodiments, methods of treating and or preventing HBV infection in a patient, comprise administering to the patient two or more arenavirus viral vectors expressing an HBV antigen or fragment thereof. In a more specific embodiment, each arenavirus viral vector expresses a different HBV antigen or fragment thereof. In other embodiments, each arenavirus viral vector expresses an HBV antigen or a derivative thereof. In some embodiments the derivative thereof is an HBV antigen fragment. In yet another embodiment provided herein are compositions that comprise two or more arena virus viral vectors each expressing a different HBV antigen or fragment thereof. In certain embodiments, the infectious arenavirus viral vector is replication-deficient (See Section 6.1(a)). In certain embodiments, the infectious arenavirus viral vector is replication-competent (See Section 6.1(b)).

[0046] In certain embodiments, the arenavirus is lymphocytic choriomeningitis virus (LCMV) or Junin virus (JUNV).

[0047] In certain embodiments, provided herein is an infectious arenavirus viral vector, wherein an arenavirus open reading frame is removed and replaced by a nucleotide sequence encoding a fusion of HBV HBs and HBc proteins or antigenic fragments thereof. In specific embodiments, the arenavirus is lymphocytic choriomeningitis virus. In specific embodiments, the open reading frame that encodes the glycoprotein of the arenavirus is deleted or functionally inactivated. In specific embodiments, the viral vector is replication-deficient. In specific embodiments, the viral vector is replication-competent. In specific embodiments, the viral vector is trisegmented. In certain embodiments, a method of treating or preventing a Hepatitis B virus infection in a patient, comprises administering to the patient the viral vector from which an arenavirus open reading frame is removed and replaced by a nucleotide sequence encoding a fusion of HBV HBs and HBc proteins or antigenic fragments thereof.

3.1 Conventions and Abbreviations

[0048]

AFP	Alpha- fetoprotein
ALT	Alanine aminotransferase
APC	Antigen presenting cells
AST	Aspartate aminotransferase
C-cell	Complementing cell line
CD4	Cluster of Differentiation 4
CD8	Cluster of Differentiation 8
CMI	Cell-mediated immunity
GS-plasmid	Plasmid expressing genome segments
HBc or HBcAg	HBV core antigen
HBe or HBeAg	Extracellular HBV core antigen
HBs or HBsAg	HBV (large) surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HRP	Horse radish peroxidase

IFN-γ	Interferon-γ
IGR	Intergenic region
JUNV	Junin virus
LCMV	Lymphocytic choriomeningitis virus
LDH	Lactate dehydrogenase
MHC	Major Histocompatibility Complex
NP	Nucleoprotein
ORF	Open reading frame
Pre-S2/S	HBV middle surface antigen
TF-plasmid	Plasmid expressing transacting factors
TNF-α	Tumor necrosis factor-a
UTR	Untranslated region
Z	Matrix Protein from LCMV

4. DESCRIPTION OF THE SEQUENCE LISTING

[0049] The following sequences are illustrative amino acid sequences and nucleotide sequences that can be used with the methods and compositions described herein. In some instances a DNA sequence is used to describe the RNA sequence of a viral genomic segment. The RNA sequence can be readily deduced from the DNA sequence. The sequences themselves may also be found in Table 3 of Section 6.10.

SEQ ID NO: 1 is the nucleotide sequence of the HBV pre-S2/S ORF.

SEQ ID NO: 2 is the nucleotide sequence of the HBV HBc ORF.

SEQ ID NO: 3 is the nucleotide sequence of the HBV HBs-HBc fusion protein ORF.

SEQ ID NO: 4 is the nucleotide sequence of the LCMV S segment expressing HBV HBs-HBc fusion protein in cDNA form. The genomic segment is RNA, the sequence in SEQ ID NO:4 is shown for DNA; however, exchanging all thymidines ("T") in SEQ ID NO:4 for uridines ("U") provides the RNA sequence.

SEQ ID NO: 5 is the nucleotide sequence of the LCMV S segment expressing the HBc ORF, in cDNA form. The genomic segment is RNA, the sequence in SEQ ID NO:5 is shown for DNA; however, exchanging all thymidines ("T") in SEQ ID NO:5 for uridines ("U") provides the RNA sequence.

SEQ ID NO: 6 is the nucleotide sequence of the LCMV S segment expressing the pre-S2/S ORF, in cDNA form. The genomic segment is RNA, the sequence in SEQ ID NO:6 is shown for DNA; however, exchanging all thymidines ("T") in SEQ ID NO:6 for uridines ("U") provides the RNA sequence.

SEQ ID NO: 7 is the lymphocytic choriomeningitis virus clone 13 segment L, complete sequence (GenBank: DQ361066.1). The genomic segment is RNA, the sequence in SEQ ID NO: 7 is shown for DNA; however, exchanging all thymidines ("T") in SEQ ID NO: 7 for uridines ("U") provides the RNA sequence.

SEQ ID NO: 8 is the amino acid sequence of an HBV HBs protein-derived epitope.

SEQ ID NO: 9 is the amino acid sequence of an HBV HBs protein-derived epitope.

SEQ ID NO: 10 is the amino acid sequence of an HBV HBc protein-derived epitope.

SEQ ID NO: 11 is the lymphocytic choriomeningitis virus segment S, complete sequence. The genomic segment is RNA, the sequence in SEQ ID NO: 11 is shown for DNA; however, exchanging all thymidines ("T") in SEQ ID NO:11 for uridines ("U") provides the RNA sequence.

SEQ ID NO: 12 is the lymphocytic choriomeningitis virus clone 13 segment S, complete sequence (GenBank: DQ361065.2). The genomic segment is RNA, the sequence in SEQ ID NO: 12 is shown for DNA; however, exchanging all thymidines ("T") in SEQ ID NO: 12 for uridines ("U") provides the RNA sequence.

SEQ ID NO: 13 is the lymphocytic choriomeningitis strain MP segment L, complete sequence. The genomic segment is RNA, the sequence in SEQ ID NO:13 is shown for DNA; however, exchanging all thymidines ("T") in SEQ ID NO:13 for uridines ("U") provides the RNA sequence.

SEQ ID NO: 14 is the lymphocytic choriomeningitis strain MP segment S, complete sequence. The genomic segment is RNA, the sequence in SEQ ID NO:14 is shown for DNA; however, exchanging all thymidines ("T") in SEQ ID NO:14 for uridines ("U") provides the RNA sequence.

SEQ ID NO: 15 is the amino acid sequence of the NP protein of the MP strain of LCMV.

SEQ ID NO: 16 is the amino acid sequence of the GP protein of the MP strain of LCMV.

SEQ ID NO: 17 is the amino acid sequence of the L protein of the MP strain of LCMV.

SEQ ID NO: 18 is the amino acid sequence of the Z protein of the MP strain of LCMV.

SEQ ID NO: 19 is Junin virus Candid #1 strain segment L, complete sequence.

SEQ ID NO: 20 is Junin virus Candid #1 strain segment S, complete sequence.

SEQ ID NO: 21 is the amino acid sequence of the NP protein of the Clone 13 strain of LCMV.

SEQ ID NO: 22 is the amino acid sequence of the GP protein of the Clone 13 strain of LCMV.

SEQ ID NO: 23 is the amino acid sequence of the L protein of the Clone 13 strain of LCMV.

SEQ ID NO: 24 is the amino acid sequence of the Z protein of the Clone 13 strain of LCMV

SEQ ID NO: 25 is the amino acid sequence of the GP protein of the WE strain of LCMV.

SEQ ID NO: 26 is the nucleotide sequence of the HBV HBe antigen.

5. BRIEF DESCRIPTION OF THE FIGURES

[0050]

Fig. 1: The genome of wild type arenaviruses consists of a short (1; ~3.4 kb) and a large (2; ~7.2 kb) RNA segment. The short segment carries ORFs encoding the nucleoprotein (3) and glycoprotein (4). The large segment encodes the RNA-dependent RNA polymerase L (5) and the matrix protein Z (6). Wild type arenaviruses can be rendered replication-deficient vaccine vectors by deleting the glycoprotein gene and inserting, instead of the glycoprotein gene, antigens of choice (7) against which immune responses are to be induced.

Figs. 2A-C: Schematic representation of the genomic organization of bi- and tri-segmented LCMV. The bi-segmented genome of wild-type LCMV consists of one S segment encoding the GP and NP and one L segment encoding the Z protein and the L protein (A). Both segments are flanked by the respective 5' and 3' UTRs. The genome of recombinant tri-segmented LCMVs (r3LCMV) consists of one L and two S segments with one position where to insert a gene of interest (here GFP) into each one of the S segments. r3LCMV-GFP^{natural} (nat) has all viral genes in their natural position (B), whereas the GP ORF in r3LCMV-GFP^{artificial} (art) is artificially juxtaposed to and expressed under control of the 3' UTR (C).

Fig. 3: Hepatitis B virus-specific CD8+ T cells, expressed as a percentage of the total CD8+B220- T cell pool in peripheral blood of C57BL/6 mice (5 mice per group) ten days after intravenous immunization with 10^5 FFU of rLCMV/HBs-HBc (group 1), rLCMV/HBc (group 3), rLCMV/Pre-S2 (group 4), or with 10^4 FFU of rLCMV/HBs-HBc (group 2). Control mice were left untreated.

Fig. 4A-B: Hepatitis B virus-specific CD8+ T cells, expressed as (A) a percentage of the total CD8+B220- T cell pool in peripheral blood or, (B) as a percentage of the circulating lymphocytes in the blood, of C57BL/6 mice (5 mice per group) eight days after intravenous immunization with 10^5 FFU of r3LCMV/HBs-HBc (group 1), r3LCMV/HBc (group 2), r3LCMV/Pre-S2 (group 3), or with 10^5 FFU of rLCMV/HBs-HBc (group 4). Control mice were left untreated.

6. DETAILED DESCRIPTION OF THE INVENTION

[0051] The present application provides immunotherapies for Hepatitis B virus infections. Provided herein are compositions and compositions for use in the treatment or prevention of infection of a subject with HBV. More specifically, provided herein are infectious arenaviruses that comprise a nucleotide sequence encoding an HBV antigen. In certain embodiments, the infectious arenavirus is replication-deficient. In certain embodiments, the infectious arenavirus is replication-competent. These viruses can be administered to a subject for the treatment or prevention of HBV infection. The generation of infectious arenavirus vectors for use with the present invention is described in more detail in Section 6.3.

[0052] Provided herein is a genetically modified arenavirus, wherein the arenavirus: is infectious;

cannot form infectious progeny virus in a non-complementary cell (i.e., a cell that does not express the functionality that is missing from the replication-deficient arenavirus and causes it to be replication-deficient);

is capable of replicating its genome and expressing its genetic information; and

encodes an HBV antigen or a fragment thereof.

[0053] A genetically modified arenavirus described herein is infectious, i.e., it can attach to a host cell and release its genetic material into the host cell. A genetically modified arenavirus described herein may be replication-deficient, i.e., the arenavirus is unable to produce further infectious progeny particles in a non-complementing cell. In particular, to create a replication-deficient arenavirus, the genome of the arenavirus is modified (e.g.,

by deletion or functional inactivation of an ORF) such that a virus carrying the modified genome can no longer produce infectious progeny viruses. A non-complementing cell is a cell that does not provide the functionality that has been eliminated from the replication-deficient arenavirus by modification of the virus genome (e.g., if the ORF encoding the GP protein is deleted or functionally inactivated, a non-complementing cell does not provide the GP protein). However, a genetically modified replication-deficient arenavirus provided herein is capable of producing infectious progeny viruses in complementing cells. Complementing cells are cells that provide (in trans) the functionality that has been eliminated from the replication-deficient arenavirus by modification of the virus genome (e.g., if the ORF encoding the GP protein is deleted or functionally inactivated, a complementing cell does provide the GP protein). Expression of the complementing functionality (e.g., the GP protein) can be accomplished by any method known to the skilled artisan (e.g., transient or stable expression). A genetically modified arenavirus described herein can amplify and express its genetic information in a cell that has been infected by the virus. A genetically modified arenavirus provided herein comprises a nucleotide sequence that encodes an HBV antigen such as but not limited to the HBV antigens described in Section 6.2.

[0054] In certain embodiments, provided herein is a genetically modified arenavirus in which an ORF of the arenavirus genome is deleted or functionally inactivated such that the resulting virus cannot produce further infectious progeny virus particles in non-complementing cells. An arenavirus particle comprising a genetically modified genome in which an ORF is deleted or functionally inactivated can be produced in complementing cells (i.e., in cells that express the arenaviral ORF that has been deleted or functionally inactivated) (see Section 6.3). The genetic material of the resulting arenavirus particles can be transferred upon infection of a host cell into the host cell, wherein the genetic material can be expressed and amplified. In addition, the genome of the genetically modified arenavirus particles provided herein encodes an HBV antigen that can be expressed in the host cell.

[0055] In certain embodiments, the ORF that encodes the glycoprotein (GP) of the arenavirus is deleted to generate a replication-deficient arenavirus for use with the present invention. In a specific embodiment, the replication-deficient arenavirus comprises a genomic segment comprising a nucleotide sequence encoding an HBV antigen. Thus, in certain embodiments, a genetically modified arenavirus particle provided herein comprises a genomic segment that a) has a deletion or functional

inactivation of an ORF that is present in the wild type form of the genomic segment; and b) encodes (either in sense or antisense) an HBV antigen (see Section 6.3).

[0056] In certain embodiments, the nucleotide sequence that is inserted into the genome of the arenavirus encoding an HBV antigen or combinations of HBV antigens is selected from :

a. a nucleotide sequence encoding an HBV pre-S2/S protein or an antigenic fragment thereof;
b. a nucleotide sequence encoding an HBV HBc protein or an antigenic fragment thereof; and
c. a nucleotide sequence encoding a fusion of HBV HBs and HBc proteins or antigenic fragments thereof;

and wherein a viral vector comprising the nucleotide sequence is capable of eliciting a T cell response against the HBV pre-S2/Sprotein, the HBV HBc protein, the fusion of HBV HBs and HBc proteins,or an antigenic fragment thereof.

[0057] In certain embodiments, the infectious arenavirus viral vector is replication-deficient (See Section 6.1(a)). In certain embodiments, the infectious arenavirus viral vector is replication-competent (See Section 6.1(b))

[0058] A detailed description of the antigens described herein is provided in Section 6.2.

[0059] In certain embodiments, the arenaviruses used according to the invention described herein can be Old World viruses, for example, Lymphocytic choriomeningitis virus (LCMV). More detailed description of the arenaviruses described herein is provided in Section 6.1. In certain embodiments, the arenaviruses used according to the invention described herein can be New World viruses.

[0060] Provided herein are nucleic acids comprising the genome of such replication-deficient arenaviruses. In certain aspects, an infectious, replication-deficient arenavirus particle comprises a genomic segment comprising a nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

[0061] Provided herein is an expression plasmid that encodes one or more components required for the generation of a viral vector described herein. Specifically, provided herein is an expression vector that encodes an LCMV S segment wherein the ORF for the GP protein has been deleted from the S segment and has been replaced with the ORF of human HBV pre-S2/S protein (e.g., having an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 1 or an amino acid sequence that is 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,

98%, 99%, or 100% identical to an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 1).

[0062] Provided herein is an expression plasmid that encodes one or more components required for the generation of a viral vector described herein. Specifically, provided herein is an expression vector that encodes an LCMV S segment wherein the ORF for the GP protein has been deleted from the S segment and has been replaced with the ORF of human HBV HBc protein (e.g., having an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 2 or an amino acid sequence that is 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 2).

[0063] Provided herein is an expression plasmid that encodes one or more components required for the generation of a viral vector described herein. Specifically, provided herein is an expression vector that encodes an LCMV S segment wherein the ORF for the GP protein has been deleted from the S segment and has been replaced with the ORF of human HBV HBs and the ORF of human HBV HBc (e.g., having an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 3 or an amino acid sequence that is 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 3).

[0064] Provided herein are kits comprising one or two of the vector plasmids described herein. In certain embodiments, provided herein is a kit that comprises a) an expression plasmid that comprises the nucleotide sequence of the S segment of an LCMV vector; b) an expression plasmid that comprises the nucleotide sequence of the L segment of an LCMV vector; and c) an expression plasmid that encodes the complementing functionality. In a specific embodiment, provided herein is a kit comprising a) an expression vector that comprises the nucleotide sequence of an LCMV S segment wherein the ORF for the GP protein has been deleted from the S segment and has been replaced with the ORF of human HBV pre-S2/S protein (e.g., having an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 1 or an amino acid sequence that is 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 1); b) an expression plasmid that comprises the nucleotide sequence of the L segment of an LCMV vector;

and c) an expression plasmid that encodes the LCMV GP protein (or a cell line that expresses LCMV GP protein).

[0065] Provided herein are kits comprising one or two of the vector plasmids described herein. In certain embodiments, provided herein is a kit that comprises a) an expression plasmid that comprises the nucleotide sequence of the S segment of an LCMV vector; b) an expression plasmid that comprises the nucleotide sequence of the L segment of an LCMV vector; and c) an expression plasmid that encodes the complementing functionality. In a specific embodiment, provided herein is a kit comprising a) an expression vector that comprises the nucleotide sequence of an LCMV S segment wherein the ORF for the GP protein has been deleted from the S segment and has been replaced with the ORF of human HBV HBc protein (e.g., having an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 2 or an amino acid sequence that is 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 2); b) an expression plasmid that comprises the nucleotide sequence of the L segment of an LCMV vector; and c) an expression plasmid that encodes the LCMV GP protein (or a cell line that expresses LCMV GP protein).

[0066] Provided herein are kits comprising one or two of the vector plasmids described herein. In certain embodiments, provided herein is a kit that comprises a) an expression plasmid that comprises the nucleotide sequence of the S segment of an LCMV vector; b) an expression plasmid that comprises the nucleotide sequence of the L segment of an LCMV vector; and c) an expression plasmid that encodes the complementing functionality. In a specific embodiment, provided herein is a kit comprising a) an expression vector that comprises the nucleotide sequence of an LCMV S segment wherein the ORF for the GP protein has been deleted from the S segment and has been replaced with the ORF of human HBV HBs and the ORF of human HBV HBc (e.g., having an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 3 or an amino acid sequence that is 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 3); b) an expression plasmid that comprises the nucleotide sequence of the L segment of an LCMV vector; and c) an expression plasmid that encodes the LCMV GP protein (or a cell line that expresses LCMV GP protein).

[0067] Also provided herein are cell lines, cultures and methods of culturing cells infected with nucleic acids, vectors, and compositions provided herein. More detailed description

of the nucleic acids, vector systems and cell lines described herein is provided in Section 6.4.

[0068] In one aspect, provided herein are such genetically modified replication-deficient arenaviruses suitable as vaccines and said arenaviruses for use in vaccination and treatment or prevention of infections by HBV. More detailed description of methods of using such arenaviruses described herein is provided in Section 6.5.

[0069] In certain embodiments, immunization with an infectious arenavirus that expresses an HBV antigen or a fragment thereof, as described herein provides a long-lasting immune response. In certain embodiments, maximal antibody levels can be achieved after two immunizations. In another embodiment, a third immunization can be administered for a boosting effect. In more specific embodiments, provided herein are administration schedules using the infectious arenavirus in a vaccination for the treatment and/or prevention of infections by HBV. A more detailed description of administration schedules using an infectious arenavirus as described herein is provided in Section 6.6. In certain embodiments, the infectious arenavirus viral vector is replication-deficient (See Section 6.1(a)). In certain embodiments, the infectious arenavirus viral vector is replication-competent (See Section 6.1(b)).

[0070] In certain embodiments, administering to a seronegative subject an infectious arenavirus expressing an HBV antigen or a fragment thereof, as described herein induces a detectable antibody titer for a minimum of at least 4 weeks. In another embodiment, administering to a subject infected with an HBV infection an infectious arenavirus expressing an HBV antigen or a fragment thereof, as described herein increases the antibody titer by at least 100%, at least 200%, at least 300%, at least 400%, at least 500%, or at least 1000%. In certain embodiments, primary antigen exposure, by first immunization with an infectious arenavirus expressing an HBV antigen, elicits a functional, (neutralizing) and minimum antibody titer of at least 50%, at least 100%, at least 200%, at least 300%, at least 400%, at least 500%, or at least 1000% of mean control sera from infection-immune human subjects. In more specific embodiments, the primary neutralizing geometric mean antibody titer increases up to a peak value of at least 1:50, at least 1:100, at least 1:200, or at least 1:1000 within at least 4 weeks post-immunization. In another embodiment, immunization with an infectious arenavirus expressing an HBV antigen or a fragment thereof, as described herein produces high titers of antibodies that last for at least 4 weeks, at least 8 weeks, at least 12 weeks, at least 6 months, at least 12 months, at least 2 years, at least 3 years, at least 4 years, or at least 5 years post-immunization following a single administration of the vaccine. In

certain embodiments, the infectious arenavirus viral vector is replication-deficient (See Section 6.1(a)). In certain embodiments, the infectious arenavirus viral vector is replication-competent (See Section 6.1(b)).

[0071] In yet another embodiment, secondary antigen exposure by second immunization with an infectious arenavirus expressing an HBV antigen or a fragment thereof increases the antibody titer by at least 100%, at least 200%, at least 300%, at least 400%, at least 500%, or at least 1000%. In another embodiment, secondary antigen exposure elicits a functional, (neutralizing) and minimum antibody titer of at least 50%, at least 100%, at least 200%, at least 300%, at least 400%, at least 500%, or at least 1000% of mean control sera from infection-immune human subjects. In more specific embodiments, the secondary neutralizing geometric mean antibody titer increases up to a peak value of at least 1:50, at least 1:100, at least 1:200, or at least 1:1000 within at least 4 weeks post-immunization. In another embodiment, a second immunization with an infectious arenavirus expressing an HBV antigen or a fragment thereof, as described herein produces high titers of antibodies that last for at least 4 weeks, at least 8 weeks, at least 12 weeks, at least 6 months, at least 12 months, at least 2 years, at least 3 years, at least 4 years, or at least 5 years post-immunization. In certain embodiments, the infectious arenavirus viral vector is replication-deficient (See Section 6.1(a)). In certain embodiments, the infectious arenavirus viral vector is replication-competent (See Section 6.1(b)).

[0072] In yet another embodiment, a third boosting immunization increases the antibody titer by at least 100%, at least 200%, at least 300%, at least 400%, at least 500%, or at least 1000%. In another embodiment, the boosting immunization elicits a functional, (neutralizing) and minimum antibody titer of at least 50%, at least 100%, at least 200%, at least 300%, at least 400%, at least 500%, or at least 1000% of mean control sera from infection-immune human subjects. In more specific embodiments, the neutralizing geometric mean antibody titer after the third boosting immunization increases up to a peak value of at least 1:50, at least 1:100, at least 1:200, or at least 1:1000 within at least 4 weeks post-immunization. In another embodiment, a third boosting immunization prolongs the antibody titer by at least 4 weeks, at least 8 weeks, at least 12 weeks, at least 6 months, at least 12 months, at least 2 years, at least 3 years, at least 4 years, or at least 5 years post-immunization.

[0073] In certain embodiments, the infectious arenavirus expressing an HBV antigen or fragment thereof, elicits a T cell independent or T cell dependent response. In other embodiments, the infectious arenavirus expressing an HBV antigen or a fragment thereof,

elicits a T cell response. In other embodiments, the infectious arenavirus expressing an HBV antigen or a fragment thereof, as described herein elicits a T helper response. In another embodiment, the infectious arenavirus expressing an HBV antigen or a fragment thereof, as described herein elicits a Th1-orientated response or a Th2-orientated response. In certain embodiments, the infectious arenavirus viral vector is replication-deficient (See Section 6.1(a)). In certain embodiments, the infectious arenavirus viral vector is replication-competent (See Section 6.1(b)).

[0074] In more specific embodiments, the Th1-orientated response is indicated by a predominance of IgG1 antibodies versus IgG2. In other embodiments the ratio of IgG1 :IgG2 is greater than 1:1, greater than 2:1, greater than 3:1, or greater than 4:1. In another embodiment the infectious arenavirus expressing an HBV antigen or a fragment thereof, as described herein is indicated by a predominance of IgG3 antibodies. In certain embodiments, the infectious arenavirus viral vector is replication-deficient (See Section 6.1(a)). In certain embodiments, the infectious arenavirus viral vector is replication-competent (See Section 6.1(b)).

[0075] In some embodiments, the infectious arenavirus expressing an HBV antigen or a fragment thereof elicits a CD8+ T cell response. In other embodiments, the infectious arenavirus expressing an HBV antigen or a fragment thereof elicits a regulatory T cell response. In more specific embodiments, the regulatory T cell response maintains immune tolerance. In another embodiment, the infectious arenavirus expressing an HBV antigen or a fragment thereof elicits both CD4+ and CD8+ T cell responses. In certain embodiments, the infectious arenavirus viral vector is replication-deficient (See Section 6.1(a)). In certain embodiments, the infectious arenavirus viral vector is replication-competent (See Section 6.1(b)).

[0076] In certain embodiments, the infectious arenavirus expressing one or more HBV antigens or fragments thereof, as described herein, elicits high titers of neutralizing antibodies. In another embodiment, the infectious arenavirus expressing two or more HBV antigens or fragments thereof, as described herein, elicits higher titers of neutralizing antibodies than expression of the protein complex components individually. In certain embodiments, the infectious arenavirus viral vector is replication-deficient (See Section 6.1(a)). In certain embodiments, the infectious arenavirus viral vector is replication-competent (See Section 6.1(b)).

[0077] In other embodiments, two or more infectious arenaviruses expressing an HBV antigen elicit high titers of neutralizing antibodies. In a more specific embodiment, two or more infectious arenaviruses expressing an HBV antigen elicit higher titers of neutralizing

antibodies than an infectious arenavirus expressing one HBV antigen or fragment thereof. In certain embodiments, the infectious arenavirus viral vector is replication-deficient (See Section 6.1(a)). In certain embodiments, the infectious arenavirus viral vector is replication-competent (See Section 6.1(b)).

[0078] In another embodiment, the infectious arenavirus expressing two, three, four, five, or more HBV antigens elicits higher titers of neutralizing antibodies than an infectious arenavirus expressing one HBV antigen or fragment thereof. In certain embodiments, the infectious arenavirus viral vector is replication-deficient (See Section 6.1(a)). In certain embodiments, the infectious arenavirus viral vector is replication-competent (See Section 6.1(b)).

6.1 Arenavirus Vectors Expressing an HBV Antigen

[0079] Arenaviruses for use with the methods and compositions provided herein can be Old World viruses, for example Lassa virus, Lymphocytic choriomeningitis virus (LCMV), Mobala virus, Mopeia virus, or Ippy virus, or New World viruses, for example Amapari virus, Flexal virus, Guanarito virus, Junin virus, Latino virus, Machupo virus, Oliveros virus, Parana virus, Pichinde virus, Pirital virus, Sabia virus, Tacaribe virus, Tamiami virus, Bear Canyon virus, or Whitewater Arroyo virus. The genetically modified arenavirus can be generated as described in Section 6.3.

[0080] The wild type arenavirus genome consists of a short (~3.4 kb) and a large (~7.2 kb) RNA segment. The short segment carries the ORFs encoding the nucleoprotein NP and glycoprotein GP genes. The large segment comprises the RNA-dependent RNA polymerase L and the matrix protein Z genes.

(a) Replication-deficient arenavirus vectors

[0081] In certain embodiments, the arenavirus vector is a replication-deficient, bisegmented arenavirus vector. In certain embodiments, the arenavirus vector is a replication-deficient, trisegmented arenavirus vector. Wild type arenaviruses can be rendered replication-deficient to generate vaccine vectors by substituting the glycoprotein gene for one or more HBV antigens, against which immune responses are to be induced.

[0082] Infectious arenavirus vectors expressing an HBV antigen, or a combination of HBV antigens as described herein, can be used to immunize (in a preventive manner) or treat

(in an immunotherapeutic manner) subjects against HBV infection. In a specific embodiment, a combination of HBs and HBc is used.

[0083] Arenavirus disease and immunosuppression in wild type arenavirus infection are known to result from unchecked viral replication. By abolishing replication, i.e., the ability to produce infectious progeny virus particles, of arenavirus vectors by deleting from their genome, e.g., the Z gene which is required for particle release, or the GP gene which is required for infection of target cells, the total number of infected cells can be limited by the inoculum administered, e.g., to a vaccine recipient, or accidentally transmitted to personnel involved in medical or biotechnological applications, or to animals. Therefore, abolishing replication of arenavirus vectors prevents pathogenesis as a result of intentional or accidental transmission of vector particles. Provided herein, one important aspect consists in exploiting the above necessity of abolishment of replication in a beneficial way for the purpose of expressing an HBV antigen. In certain embodiments, an arenavirus particle is rendered replication deficient by genetic modification of its genome. Such modifications to the genome can include:

deletion of an ORF (e.g., the ORF encoding the GP, NP, L, or Z protein);

functional inactivation of an ORF (e.g., the ORF encoding the GP, NP, L, or Z protein).

For example, this can be achieved by introducing a missense or a nonsense mutation.;

change of the sequence of the ORF (e.g., the exchange of an SIP cleavage site with the cleavage site of another protease);

mutagenesis of one of the 5' or 3' termini of one of the genomic segments;

mutagenesis of an intergenic region (i.e., of the L or the S genomic segment).

[0084] In certain embodiments, an infectious arenavirus expressing an HBV antigen described herein is a Lymphocytic choriomeningitis virus (LCMV) wherein the S segment of the virus is modified by substituting the ORF encoding the GP protein with an ORF encoding an HBV antigen.

[0085] In certain embodiments, a wild type arenavirus vector genome (FIG. 1) can be designed to retain at least the essential regulatory elements on the 5' and 3' untranslated regions (UTRs) of both segments, and/or also the intergenic regions (IGRs). Without being bound by theory, the minimal transacting factors for gene expression in infected cells remain in the vector genome as ORFs that can be expressed, yet they can be placed differently in the genome and can be placed under control of a different promoter than naturally, or can be expressed from internal ribosome entry sites. In certain embodiments, the nucleic acid encoding an HBV antigen is transcribed from one of the endogenous

arenavirus promoters (i.e., 5' UTR, 3' UTR of the S segment, 5' UTR, 3' UTR of the L segment). In other embodiments, the nucleic acid encoding an HBV antigen is expressed from a heterologous introduced promoter sequences that can be read by the viral RNA-dependent RNA polymerase, by cellular RNA polymerase I, RNA polymerase II or RNA polymerase III, such as duplications of viral promoter sequences that are naturally found in the viral UTRs, the 28S ribosomal RNA promoter, the beta-actin promoter or the 5S ribosomal RNA promoter, respectively. In certain embodiments ribonucleic acids coding for HBV antigens are transcribed and translated either by themselves or as read-through by fusion to arenavirus protein ORFs, and expression of proteins in the host cell may be enhanced by introducing in the viral transcript sequence at the appropriate place(s) one or more, e.g., two, three or four, internal ribosome entry sites.

[0086] In certain embodiments, for use with the compositions and methods provided herein is a tri-segmented arenavirus particle comprising one L segment and two S segments in which (i) an ORF is in a position other than the wild-type position of the ORF; and (ii) an ORF encoding GP or NP has been removed or functionally inactivated, such that the resulting virus cannot produce further infectious progeny virus particles. In a specific embodiment, one ORF is removed and replaced with a heterologous ORF (e.g., encoding an HBV antigen) from an organism other than an arenavirus. In another specific embodiment, two ORFs are removed and replaced with a heterologous ORF from an organism other than an arenavirus. In other specific embodiments, three ORFs are removed and replaced with a heterologous ORF (e.g., encoding an HBV antigen) from an organism other than an arenavirus. In specific embodiments, the ORF encoding GP is removed and replaced with a heterologous ORF (e.g., encoding an HBV antigen) from an organism other than an arenavirus. In other specific embodiments, the ORF encoding NP is removed and replaced with a heterologous ORF (e.g., encoding an HBV antigen) from an organism other than an arenavirus. In yet more specific embodiments, the ORF encoding NP and the ORF encoding GP are removed and replaced with one or two heterologous ORFs (*e.g.*, encoding one or two HBV antigens) from an organism other than an arenavirus particle. Thus, in certain embodiments the tri-segmented arenavirus particle comprises (i) one L segment and two S segments; (ii) an ORF in a position other than the wild-type position of the ORF; (iii) one or more heterologous ORFs (e.g., encoding one or more HBV antigens) from an organism other than an arenavirus.

[0087] In certain embodiments, for use with the compositions and methods provided herein is a tri-segmented arenavirus particle comprising two L segments and one S segment in which (i) an ORF is in a position other than the wild-type position of the ORF;

and (ii) an ORF encoding the Z protein, and/or the L protein has been removed or functionally inactivated, such that the resulting virus cannot produce further infectious progeny virus particle. In a specific embodiment, one ORF is removed and replaced with a heterologous ORF (e.g., encoding an HBV antigen) from an organism other than an arenavirus. In another specific embodiment, two ORFs are removed and replaced with a heterologous ORF (e.g., encoding an HBV antigen) from an organism other than an arenavirus. In specific embodiments, the ORF encoding the Z protein is removed and replaced with a heterologous ORF (e.g., encoding an HBV antigen) from an organism other than an arenavirus. In other specific embodiments, the ORF encoding the L protein is removed and replaced with a heterologous ORF (e.g., encoding an HBV antigen) from an organism other than an arenavirus. In yet more specific embodiments, the ORF encoding the Z protein and the ORF encoding the L protein is removed and replaced with a heterologous ORF (e.g., encoding an HBV antigen) from an organism other than an arenavirus particle. Thus, in certain embodiments the tri-segmented arenavirus particle comprises (i) two L segments and one S segment; (ii) an ORF in a position other than the wild-type position of the ORF; (iii) a heterologous ORF (e.g., encoding an HBV antigen) from an organism other than an arenavirus.

[0088] Thus, in certain embodiments, the tri-segmented arenavirus particle for use with the compositions and methods provided herein comprises a tri-segmented arenavirus particle *(i.e.,* one L segment and two S segments or two L segments and one S segment) that i) is engineered to carry an ORF in a non-natural position; ii) an ORF encoding GP, NP, Z protein, or L protein is removed; iii) the ORF that is removed is replaced with one or more heterologous ORFs (e.g., encoding one or more HBV antigens) from an organism other than an arenavirus.

[0089] In certain embodiments, the vector generated to encode one or more HBV antigens may be based on a specific strain of LCMV. Strains of LCMV include Clone 13, MP strain, Arm CA 1371, Arm E-250, WE, UBC, Traub, Pasteur, 810885, CH-5692, Marseille #12, HP65-2009, 200501927, 810362, 811316, 810316, 810366, 20112714, Douglas, GR01, SN05, CABN and their derivatives. In certain embodiments, the vector generated to encode one or more HBV antigens may be based on LCMV Clone 13. In other embodiments, the vector generated to encode one or more HBV antigens may be based on LCMV MP strain. The sequence of the S segment of LCMV Clone 13 is listed as SEQ ID NO: 12. In certain embodiments, the sequence of the S segment of LCMV Clone 13 is the sequence set forth in SEQ ID NO: 11. The sequence of the L segment of LCMV Clone 13 is listed as SEQ ID NO: 7. The sequence of the S segment of LCMV

strain MP is listed as SEQ ID NO: 14. The sequence of the L segment of LCMV strain MP is listed as SEQ ID NO: 13.

[0090] In certain embodiments, the vector generated to encode one or more HBV antigens may be based on a specific strain of Junin virus. Strains of Junin virus include vaccine strains XJ13, XJ#44, and Candid#1 as well as IV4454, a human isolate. In certain embodiments, the vector generated to encode one or more HBV antigens is based on Junin virus Candid #1 strain.

[0091] In certain embodiments, an infectious, replication-deficient arenavirus particle in the context of the invention comprises a nucleotide sequence or fragment thereof selected from SEQ ID NO: 13, SEQ ID NO: 14, or a combination thereof.

[0092] In certain embodiments, described herein is an infectious, replication-deficient arenavirus particle comprising a nucleotide sequence, or a combination of nucleotide sequences, selected from the group consisting of :

- a nucleotide sequence encoding a Hepatitis B virus pre-S2/S protein or an antigenic fragment thereof;
- a nucleotide sequence encoding a Hepatitis B virus HBc protein or an antigenic fragment thereof; and
- a nucleotide sequence encoding a fusion of Hepatitis B virus HBs and HBc proteins or antigenic fragments thereof;

and wherein the arenavirus particle is capable of eliciting a T cell response against the HBV pre-S2/S protein, the HBc protein, the fusion of HBV HBs and HBc proteins, or an antigenic fragment thereof.

[0093] In certain embodiments, the infectious, replication-deficient arenavirus vector is trisegmented.

(b) Replication-competent trisegmented arenavirus vectors

[0094] In certain embodiments, for use with the compositions and methods provided herein is a replication-competent, trisegmented arenavirus vector. In certain embodiments, the arenavirus vector is a tri-segmented arenavirus particle comprising one L segment and two S segments or two L segments and one S segment that does not recombine into a replication-competent bi-segmented arenavirus particle.

[0095] In certain embodiments, an infectious arenavirus expressing an HBV antigen for use with the compositions and methods described herein is engineered to carry a viral ORF in a position other than the wild-type position of the ORF. In some embodiments,

the arenavirus genomic segment is selected from the group consisting of: (i) an S segment, wherein the ORF encoding the NP is under control of an arenavirus 5' UTR; (ii) an S segment, wherein the ORF encoding the Z protein is under control of an arenavirus 5' UTR; (iii) an S segment, wherein the ORF encoding the L protein is under control of an arenavirus 5' UTR; (iv) an S segment, wherein the ORF encoding the GP is under control of an arenavirus 3' UTR; (v) an S segment, wherein the ORF encoding the L protein is under control of an arenavirus 3' UTR; (vi) an S segment, wherein the ORF encoding the Z protein is under control of an arenavirus 3' UTR; (vii) an L segment, wherein the ORF encoding the GP is under control of an arenavirus 5' UTR; (viii) an L segment, wherein the ORF encoding the NP is under control of an arenavirus 5' UTR; (ix) an L segment, wherein the ORF encoding the L protein is under control of an arenavirus 5' UTR; (x) an L segment, wherein the ORF encoding the GP is under control of an arenavirus 3' UTR; (xi) an L segment, wherein the ORF encoding the NP is under control of an arenavirus 3' UTR; and (xii) an L segment, wherein the ORF encoding the Z protein is under control of an arenavirus 3' UTR.

[0096] In some embodiments, the arenavirus 3' UTR is the 3' UTR of the arenavirus S segment or the arenavirus L segment. In certain embodiments, the arenavirus 5' UTR is the 5' UTR of the arenavirus S segment or the arenavirus L segment.

[0097] For use with the compositions and methods, provided herein are tri-segmented arenavirus particles with rearrangements of their ORFs. In one aspect, for use with the compositions and methods, provided herein is a tri-segmented arenavirus particle comprising one L segment and two S segments or two L segments and one S segment. In certain embodiments, the tri-segmented arenavirus particle does not recombine into a replication competent bi-segmented arenavirus particle. In specific embodiments, the tri-segmented arenavirus particle comprises an ORF in a position other than the wild-type position of the ORF. In yet another specific embodiment, the tri-segmented arenavirus particle comprises all four arenavirus ORFs. Thus, in certain embodiments, the tri-segmented arenavirus particle is replication competent and infectious. Figure 2 shows exemplary schematic representations of the genomic organization of a replication-competent trisegmented LCMV vector (Figs. 2B-C). Figure 2C shows an exemplary schematic representation of the genomic organization of replication-competent trisegmented LCMV vector which cannot recombine into a replication-competent bisegmented arenavirus particle. In comparison, Figure 2A shows the wildtype bisegmented LCMV vector.

[0098] In certain embodiments, the ORF encoding GP, NP, Z protein, or the L protein of the tri-segmented arenavirus particle described herein can be under the control of an arenavirus 3' UTR or an arenavirus 5' UTR. In more specific embodiments, the tri-segmented arenavirus 3' UTR is the 3' UTR of an arenavirus S segment(s). In another specific embodiment, the tri-segmented arenavirus 3' UTR is the 3' UTR of an arenavirus L segment(s). In more specific embodiments, the tri-segmented arenavirus 5' UTR is the 5' UTR of an arenavirus S segment(s). In other specific embodiments, the 5' UTR is the 5' UTR of an arenavirus L segment(s).

[0099] In other embodiments, the ORF encoding GP, NP, Z protein, or the L protein of an tri-segmented arenavirus particle described herein can be under the control of the arenavirus conserved terminal sequence element (the 5'- and 3'-terminal 19-20-nt regions) (see *e.g.,* Perez & de la Torre, 2003, J Virol. 77(2): 1184-1194).

[0100] In certain embodiments, the ORF encoding GP, NP, Z protein or the L protein of the tri-segmented arenavirus particle can be under the control of the promoter element of the 5' UTR (see *e.g.,* Albarino et al., 2011, J Virol., 85(8):4020-4). In another embodiment, the ORF encoding GP, NP, Z protein, L protein of the tri-segmented arenavirus particle can be under the control of the promoter element of the 3' UTR (see *e.g.,* Albarino et al., 2011, J Virol., 85(8):4020-4). In more specific embodiments, the promoter element of the 5' UTR is the 5' UTR promoter element of the S segment(s) or the L segment(s). In another specific embodiment, the promoter element of the 3' UTR is the 3' UTR the promoter element of the S segment(s) or the L segment(s).

[0101] In certain embodiments, the ORF that encodes GP, NP, Z protein or the L protein of the tri-segmented arenavirus particle can be under the control of a truncated arenavirus 3' UTR or a truncated arenavirus 5' UTR (see *e.g.,* Perez & de la Torre, 2003, J Virol. 77(2): 1184-1194; Albarino et al., 2011, J Virol., 85(8):4020-4). In more specific embodiments, the truncated 3' UTR is the 3' UTR of the arenavirus S segment or L segment. In more specific embodiments, the truncated 5' UTR is the 5' UTR of the arenavirus S segment(s) or L segment(s).

[0102] In one aspect, for use with the compositions and methods, provided herein is a tri-segmented arenavirus particle comprising one L segment and two S segments. In certain embodiments, propagation of the tri-segmented arenavirus particle comprising one L segment and two S segments does not result in a replication-competent bi-segmented viral particle. In specific embodiments, propagation of the tri-segmented arenavirus particle comprising one L segment and two S segments does not result in a replication-competent bi-segmented viral particle after at least 10 days, at least 20 days, at least 30

days, at least 40 days, at least 50 days, at least 60 days, at least 70 days, at least 80 days, at least 90 days, or at least 100 days of persistent infection in mice lacking type I interferon receptor, type II interferon receptor and recombination activating gene (RAG1), and having been infected with 10^4 PFU of the tri-segmented arenavirus particle. In other embodiments, propagation of the tri-segmented arenavirus particle comprising one L segment and two S segments does not result in a replication-competent bi-segmented viral particle after at least 10 passages, at least 20 passages, at least 30 passages, at least 40 passages, or at least 50 passages.

[0103] The tri-segmented arenavirus particle with all viral genes in their respective wild-type position is known in the art *(e.g.,* Emonet et al., 2011 J. Virol., 85(4):1473; Popkin et al., 2011, J. Virol, 85(15):7928). In particular, the tri-segmented arenavirus genome consists of one L segment and two S segments, in which a heterologous ORF *(e.g.,* a GFP) is inserted into one position on each S segment. More specifically, one S segment encodes GP and GFP, respectively. The other S segment encodes GFP and NP, respectively. The L segment encodes the L protein and Z protein. All segments are flanked by the respective 5' and 3' UTRs.

[0104] In certain embodiments, inter-segmental recombination of the two S segments of the tri-segmented arenavirus particle for use with the compositions and methods provided herein, that unities the two arenaviral ORFs on one instead of two separate segments results in a non functional promoter *(i.e.,* a genomic segment of the structure: 5' UTR----------5' UTR or a 3' UTR----------3' UTR), wherein each UTR forming one end of the genome is an inverted repeat sequence of the other end of the same genome.

[0105] In certain embodiments, the tri-segmented arenavirus particle comprising one L segment and two S segments has been engineered to carry an arenavirus ORF in a position other than the wild-type position of the ORF. In other embodiments, the tri-segmented arenavirus particle comprising one L segment and two S segments has been engineered to carry two arenavirus ORFs, or three arenavirus ORFs, or four arenavirus ORFs, or five arenavirus ORFs, or six arenavirus ORFs in a position other than the wild-type position. In specific embodiments, the tri-segmented arenavirus particle comprising one L segment and two S segments comprises a full complement of all four arenavirus ORFs. Thus, in some embodiments, the tri-segmented arenavirus particle is an infectious and replication competent tri-segmented arenavirus particle. In specific embodiments, the two S segments of the tri-segmented arenavirus particle have been engineered to carry one of their ORFs in a position other than the wild-type position. In more specific embodiments, the two S segments comprise a full complement of the S segment ORF's.

In certain specific embodiments, the L segment has been engineered to carry an ORF in a position other than the wild-type position or the L segment can be the wild-type genomic segment.

[0106] In certain embodiments, one of the two S segments can be:

i. an arenavirus S segment, wherein the ORF encoding the Z protein is under control of an arenavirus 5' UTR;
ii. an arenavirus S segment, wherein the ORF encoding the L protein is under control of an arenavirus 5' UTR;
iii. an arenavirus S segment, wherein the ORF encoding the NP is under control of an arenavirus 5' UTR;
iv. an arenavirus S segment, wherein the ORF encoding the GP is under control of an arenavirus 3' UTR;
v. an arenavirus S segment, wherein the ORF encoding the L is under control of an arenavirus 3' UTR; and
vi. an arenavirus S segment, wherein the ORF encoding the Z protein is under control of an arenavirus 3' UTR.

[0107] In certain embodiments, the tri-segmented arenavirus particle comprising one L segment and two S segments can comprise a duplicate ORF *(i.e.,* two wild-type S segment ORFs *e.g.,* GP or NP). In specific embodiments, the tri-segmented arenavirus particle comprising one L segment and two S segments can comprise one duplicate ORF *(e.g.,* (GP, GP)) or two duplicate ORFs *(e.g.,* (GP, GP) and (NP, NP)).

[0108] Table 1A, below, is an exemplary illustration of the genome organization of a tri-segmented arenavirus particle comprising one L segment and two S segments, wherein intersegmental recombination of the two S segments in the tri-segmented arenavirus genome does not result in a replication-competent bi-segmented viral particle and abrogates arenaviral promoter activity *(i.e.,* the resulting recombined S segment is made up of two 3'UTRs instead of a 3' UTR and a 5' UTR).

Table 1A

Tri-segmented arenavirus particle comprising one L segment and two S segments Position 1 is under the control of an arenavirus S segment 5' UTR; Position 2 is under the control of an arenavirus S segment 3' UTR; Position 3 is under the control of an arenavirus S segment 5' UTR; Position 4 under the control of an arenavirus S segment 3' UTR; Position 5 is under the control of an arenavirus L segment 5' UTR; Position 6 is under the control of an arenavirus L segment 3' UTR.					
Position 1	**Position 2**	**Position 3**	**Position 4**	**Position 5**	**Position 6**
*ORF	GP	*ORF	NP	Z	L
*ORF	NP	*ORF	GP	Z	L
*ORF	NP	*ORF	GP	L	Z
*ORF	NP	*ORF	Z	L	GP
*ORF	NP	Z	GP	*ORF	Z
*ORF	NP	Z	GP	Z	*ORF
*ORF	NP	*ORF	L	Z	GP
*ORF	L	*ORF	NP	Z	GP
*ORF	L	Z	NP	*ORF	GP
*ORF	L	*ORF	GP	Z	NP
*ORF	L	Z	GP	*ORF	NP
*ORF	Z	L	NP	*ORF	GP
*ORF	Z	*ORF	GP	L	NP
*ORF	Z	L	GP	*ORF	NP
L	GP	*ORF	NP	*ORF	Z
L	GP	*ORF	Z	*ORF	NP
L	*ORF	Z	GP	*ORF	NP
L	GP	*ORF	NP	*ORF	Z
L	GP	*ORF	Z	*ORF	NP
L	*ORF	Z	NP	*ORF	GP
L	NP	*ORF	Z	*ORF	GP

Tri-segmented arenavirus particle comprising one L segment and two S segments Position 1 is under the control of an arenavirus S segment 5' UTR; Position 2 is under the control of an arenavirus S segment 3' UTR; Position 3 is under the control of an arenavirus S segment 5' UTR; Position 4 under the control of an arenavirus S segment 3' UTR; Position 5 is under the control of an arenavirus L segment 5' UTR; Position 6 is under the control of an arenavirus L segment 3' UTR.

Position 1	Position 2	Position 3	Position 4	Position 5	Position 6
L	NP	Z	*ORF	GP	*ORF
L	*ORF	Z	*ORF	GP	NP
L	NP	*ORF	Z	*ORF	GP
L	*ORF	Z	NP	*ORF	GP
L	Z	*ORF	GP	*ORF	NP
L	Z	*ORF	NP	*ORF	GP
Z	GP	*ORF	NP	*ORF	L
Z	GP	*ORF	L	*ORF	NP
Z	*ORF	L	GP	*ORF	NP
Z	GP	*ORF	NP	*ORF	L
Z	GP	*ORF	L	*ORF	NP
Z	*ORF	L	NP	*ORF	GP
Z	NP	*ORF	GP	*ORF	L
Z	NP	*ORF	L	*ORF	GP
Z	*ORF	L	GP	*ORF	NP
Z	NP	*ORF	GP	*ORF	L
Z	NP	*ORF	L	*ORF	GP
Z	*ORF	L	NP	*ORF	GP
Z	L	*ORF	GP	*ORF	NP

*ORF indicates that a heterologous ORF, for example, a heterologous ORF encoding an HBV antigen, has been inserted.

[0109] In certain embodiments, the IGR between position one and position two can be an arenavirus S segment or L segment IGR; the IGR between position three and four can be an arenavirus S segment or L segment IGR; and the IGR between the position five and six can be an arenavirus L segment IGR. In a specific embodiment, the IGR between position one and position two can be an arenavirus S segment IGR; the IGR between position three and four can be an arenavirus S segment IGR; and the IGR between the position five and six can be an arenavirus L segment IGR. In certain embodiments, other combinations are also possible. For example, a tri-segmented arenavirus particle comprising one L segment and two S segments, wherein intersegmental recombination of the two S segments in the tri-segmented arenavirus genome does not result in a replication-competent bi-segmented viral particle and abrogates arenaviral promoter activity (i.e., the resulting recombined S segment is made up of two 5'UTRs instead of a 3' UTR and a 5' UTR).

[0110] In certain embodiments, intersegmental recombination of an S segment and an L segment in the tri-segmented arenavirus particle comprising one L segment and two S segments, restores a functional segment with two viral genes on only one segment instead of two separate segments. In other embodiments, intersegmental recombination of an S segment and an L segment in the tri-segmented arenavirus particle comprising one L segment and two S segments does not result in a replication-competent bi-segmented viral particle.

[0111] In one aspect, for use with the compositions and methods, provided herein is a tri-segmented arenavirus particle comprising two L segments and one S segment. In certain embodiments, propagation of the tri-segmented arenavirus particle comprising two L segments and one S segment does not result in a replication-competent bi-segmented viral particle. In specific embodiments, propagation of the tri-segmented arenavirus particle comprising two L segments and one S segment does not result in a replication-competent bi-segmented viral particle after at least 10 days, at least 20 days, at least 30 days, at least 40 days, or at least 50 days, at least 60 days, at least 70 days, at least 80 days, at least 90 days, at least 100 days of persistent infection in mice lacking type I interferon receptor, type II interferon receptor and recombination activating gene (RAG1), and having been infected with 10^4 PFU of the tri-segmented arenavirus particle. In other embodiments, propagation of the tri-segmented arenavirus particle comprising two L segments and one S segment does not result in a replication-competent bi-segmented viral particle after at least 10 passages, 20 passages, 30 passages, 40 passages, or 50 passages.

[0112] In certain embodiments, inter-segmental recombination of the two L segments of the tri-segmented arenavirus particle for use with the compositions and methods provided herein, that unities the two arenaviral ORFs on one instead of two separate segments results in a non functional promoter (i.e., a genomic segment of the structure: 5' UTR----------5' UTR or a 3' UTR----------3' UTR), wherein each UTR forming one end of the genome is an inverted repeat sequence of the other end of the same genome.

[0113] In certain embodiments, the tri-segmented arenavirus particle comprising two L segments and one S segment has been engineered to carry an arenavirus ORF in a position other than the wild-type position of the ORF. In other embodiments, the tri-segmented arenavirus particle comprising two L segments and one S segment has been engineered to carry two arenavirus ORFs, or three arenavirus ORFs, or four arenavirus ORFs, or five arenavirus ORFs, or six arenavirus ORFs in a position other than the wild-type position. In specific embodiments, the tri-segmented arenavirus particle comprising two L segments and one S segment comprises a full complement of all four arenavirus ORFs. Thus, in some embodiments, the tri-segmented arenavirus particle is an infectious and replication competent tri-segmented arenavirus particle. In specific embodiments, the two L segments of the tri-segmented arenavirus particle have been engineered to carry one of their ORFs in a position other than the wild-type position. In more specific embodiments, the two L segments comprise a full complement of the L segment ORF's. In certain specific embodiments, the S segment has been engineered to carry one of their ORFs in a position other than the wild-type position or the S segment can be the wild-type genomic segment.

[0114] In certain embodiments, one of the two L segments can be:

i. an L segment, wherein the ORF encoding the GP is under control of an arenavirus 5' UTR;
ii. an L segment, wherein the ORF encoding NP is under control of an arenavirus 5' UTR;
iii. an L segment, wherein the ORF encoding the L protein is under control of an arenavirus 5' UTR;
iv. an L segment, wherein the ORF encoding the GP is under control of an arenavirus 3' UTR;
v. an L segment, wherein the ORF encoding the NP is under control of an arenavirus 3' UTR; and
vi. an L segment, wherein the ORF encoding the Z protein is under control of an arenavirus 3' UTR.

[0115] In certain embodiments, the tri-segmented arenavirus particle comprising two L segments and S segments can comprise a duplicate ORF *(i.e.,* two wild-type L segment ORFs *e.g.,* Z protein or L protein). In specific embodiments, the tri-segmented arenavirus particle comprising two L segments and one S segment can comprise one duplicate ORF *(e.g.,* (Z protein, Z protein)) or two duplicate ORFs *(e.g.,* (Z protein, Z protein) and (L protein, L protein)).

[0116] Table 2A, below, is an exemplary illustration of the genome organization of a tri-segmented arenavirus particle comprising two L segments and one S segment, wherein intersegmental recombination of the two L segments in the tri-segmented arenavirus genome does not result in a replication-competent bi-segmented viral particle and abrogates arenaviral promoter activity (*i.e.,* the resulting L segment is made up of two 3'UTRs instead of a 3' UTR and a 5' UTR). Based on Table 3 similar combinations could be predicted for generating an arenavirus particle made up of two 5' UTRs instead of a 3' UTR and a 5' UTR.

Table 2A

Tri-segmented arenavirus particle comprising two L segments and one S segment					
Position 1	**Position 2**	**Position 3**	**Position 4**	**Position 5**	**Position 6**
*ORF	Z	*ORF	L	NP	GP
*ORF	Z	*ORF	L	GP	NP
*ORF	Z	GP	L	*ORF	NP
*ORF	Z	*ORF	GP	NP	L
*ORF	Z	GP	NP	*ORF	L
*ORF	Z	NP	GP	*ORF	L
*ORF	L	*ORF	Z	NP	GP
*ORF	L	*ORF	Z	GP	NP
*ORF	L	*ORF	GP	NP	Z
*ORF	L	GP	Z	*ORF	NP
*ORF	L	NP	Z	*ORF	GP
*ORF	L	GP	NP	*ORF	Z
*ORF	L	NP	GP	*ORF	Z

*ORF	GP	*ORF	L	NP	Z
*ORF	GP	NP	L	*ORF	Z
*ORF	GP	*ORF	Z	NP	L
*ORF	GP	NP	Z	*ORF	L
*ORF	NP	*ORF	L	GP	Z
*ORF	NP	GP	L	*ORF	Z
*ORF	NP	GP	Z	*ORF	L
*ORF	NP	*ORF	Z	GP	L
*ORF	L	*ORF	Z	NP	GP
*ORF	L	*ORF	Z	GP	NP
*ORF	L	*ORF	NP	GP	Z
*ORF	L	*ORF	GP	NP	Z
*ORF	L	NP	Z	*ORF	GP
*ORF	Z	*ORF	GP	NP	L
*ORF	Z	GP	L	*ORF	NP
*ORF	Z	NP	GP	*ORF	L
*ORF	Z	GP	NP	*ORF	L
*ORF	GP	*ORF	L	Z	NP
*ORF	GP	*ORF	Z	GP	L
*ORF	GP	NP	L	*ORF	Z
GP	L	*ORF	Z	*ORF	NP
GP	L	*ORF	NP	*ORF	Z
GP	Z	*ORF	L	*ORF	NP
GP	Z	*ORF	NP	*ORF	L
GP	NP	*ORF	Z	*ORF	L
NP	L	*ORF	Z	*ORF	GP
NP	L	*ORF	GP	*ORF	Z

*Position 1 is under the control of an arenavirus L segment 5' UTR; position 2 is under the control of an arenavirus L segment 3' UTR; position 3 is under the control of an arenavirus L segment 5' UTR; position 4 is under the control of an arenavirus L segment 3' UTR; position 5 is under the control of an arenavirus S segment 5' UTR; position 6 is under the control of an arenavirus S segment 3' UTR.
* ORF indicates that a heterologous ORF, for example, a heterologous ORF encoding an HBV antigen, has been inserted.

[0117] In certain embodiments, the IGR between position one and position two can be an arenavirus S segment or L segment IGR; the IGR between position three and four can be an arenavirus S segment or L segment IGR; and the IGR between the position five and six can be an arenavirus S segment IGR. In a specific embodiment, the IGR between position one and position two can be an arenavirus L segment IGR; the IGR between position three and four can be an arenavirus L segment IGR; and the IGR between the position five and six can be an arenavirus S segment IGR. In certain embodiments, other combinations are also possible.

[0118] In certain embodiments intersegmental recombination of an L segment and an S segment from the tri-segmented arenavirus particle comprising two L segments and one S segment restores a functional segment with two viral genes on only one segment instead of two separate segments. In other embodiments, intersegmental recombination of an L segment and an S segment in the the tri-segmented arenavirus particle comprising two L segments and one S segment does not result in a replication-competent bi-segmented viral particle.

[0119] In certain embodiments, the tri-segmented arenavirus particle as described herein is an infectious and replication competent arenavirus particle. In specific embodiments, the arenavirus particle described herein is attenuated. In a particular embodiment, the tri-segmented arenavirus particle is attenuated such that the virus remains, at least partially, replication-competent and can replicate *in vivo*, but can only generate low viral loads resulting in subclinical levels of infection that are non-pathogenic. Such attenuated viruses can be used as an immunogenic composition. In other embodiments, the arenavirus particle is infectious but unable to produce further infectious progeny in non-complementing cells.

[0120] In certain embodiments, the arenavirus genomic segment, and the respective arenavirus particle or tri-segmented arenavirus particle can comprise a heterologous ORF. In other embodiments, the arenavirus genomic segment and the respective

arenavirus particle or tri-segmented arenavirus particle can comprise a gene of interest. In more specific embodiments, the heterologous ORF or the gene of interest encodes an antigen. In more specific embodiments, the heterologous ORF or the gene or interest encodes an HBV antigen or an antigenic fragment thereof (see Section 6.2).

[0121] In certain embodiments, the arenavirus genomic segment, the arenavirus particle or the tri-segmented arenavirus particle can comprise one or more heterologous ORFs or one or more genes of interest. In other embodiments, the arenavirus genomic segment, the arenavirus particle or the tri-segmented arenavirus particle can comprise at least one heterologous ORF, at least two heterologous ORFs, at least three heterologous ORFs, or more heterologous ORFs. In other embodiments, the arenavirus particle or the tri-segmented arenavirus particle comprises at least one gene of interest, at least two genes of interest, at least three genes of interest, or more genes of interest. In more specific embodiments, the one or more heterologous ORFs or the genes of interest encode one or more HBV antigens or antigenic fragments thereof (see Section 6.2).

[0122] In certain embodiments, an infectious arenavirus expressing an HBV antigen described herein is a tri-segmented arenavirus particle comprising one L segment and two S segments. In certain embodiments, an infectious arenavirus expressing an HBV antigen described herein is a tri-segmented arenavirus particle comprising two L segments and one S segment.

6.2 HBV Antigens

[0123] In certain embodiments, antigens for use with the methods and compositions described herein are HBV antigens.

[0124] In certain embodiments, the ORFs of two or more HBV antigens described are transcribed as a single transcript.

[0125] In certain embodiments, any genotype or subgenotype of human HBV or any clinical isolate of human HBV can be used with the present invention to obtain the antigens for generation of the arenaviral vectors described herein. Such HBV genotypes and subgenotypes include genotypes A-J, and subgenotypes A1-A6, B1-B4, C1-C6, D1-D7, and F1-F4.

[0126] In certain embodiments, the HBV antigen can be an HBV antigen ortholog, e.g., a mammalian (i.e., non-human primate, pig, dog, cat, or horse) HBV antigen.

(a) pre-S2/S protein antigens

[0127] In certain embodiments, the antigen is the HBV pre-S2/S protein or a fragment thereof. In certain embodiments, the antigen is a fragment of at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150 or more amino acids of HBV pre-S2/S protein. In certain embodiments, the antigen is an antigenic fragment of HBV pre-S2/S protein. In certain embodiments, the antigen is encoded by a nucleic acid sequence that is 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 1. In certain embodiments, the antigen comprises an amino acid sequence that is 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 1.

(b) HBc protein antigens

[0128] In certain embodiments, the antigen is the HBV HBc protein or a fragment thereof. In certain embodiments, the antigen is a fragment of at least 10, 15, 20, 25, 50, 75, 100, 125, 150 or more amino acids of the HBV HBc protein. In certain embodiments, the antigen is an antigenic fragment of HBc. In certain embodiments, the antigen is encoded by a nucleic acid sequence that is 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2. In certain embodiments, the antigen comprises an amino acid sequence that is 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 2.

(c) HBs protein antigens

[0129] In certain examples, not in the context of the invention, the antigen is the HBV HBs protein or a fragment thereof. In certain examples, the antigen is a fragment of at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or more amino acids of the HBV HBs protein. In certain examples, the antigen is an antigenic fragment of HBs.

[0130] In certain examples, the antigen is the HBV HBs small polypeptide (*e.g.* "S") or a fragment thereof. In certain examples, the antigen is the HBV HBs medium polypeptide (*e.g.*, "pre-S2/S") or a fragment thereof. In certain examples, the antigen is the HBV HBs

large polypeptide *(e.g.,* "pre-S1/pre-S2/S") or a fragment thereof. In certain examples, the antigen is a fragment of at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150 or more amino acids of the HBV HBs small polypeptide. In certain examples, the antigen is a fragment of at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150 or more amino acids of the HBV HBs medium polypeptide. In certain examples, the antigen is a fragment of at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 100, 150, 200, 250, 300, 350 or more amino acids of the HBV HBs large polypeptide.

(d) HBs and HBc fusion proteins

[0131] In certain embodiments, the antigen is a fusion protein of the HBV HBs and HBc proteins or antigenic fragments thereof. In certain embodiments, the antigen is a fragment of at least 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225 or more amino acids of a fusion protein of HBs and HBc. In certain embodiments, the antigen is encoded by a nucleic acid sequence that is 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 3. In certain embodiments, the antigen comprises an amino acid sequence that is 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 3.

(e) HBe protein antigens

[0132] In certain examples, not in the context of the invention, the antigen is the HBV HBe protein or a fragment thereof. In certain examples, the antigen is a fragment of at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150 or more amino acids of the HBV HBe protein. In certain examples, the antigen is an antigenic fragment of HBe. In certain examples, the antigen is encoded by a nucleic acid sequence that is 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 26. In certain examples, the antigen comprises an amino acid sequence that is 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 26.

(f) Polymerase protein antigens

[0133] In certain examples, not in the context of the invention, the antigen is an HBV polymerase protein or antigenic fragment thereof. In certain examples, the antigen is a fragment of at least 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 300, 400, 500, 600, 700 or more amino acids of an HBV polymerase protein.

[0134] Nucleic acid sequences encoding an HBV antigen can be introduced in the genome of an infectious arenavirus by substitution of the nucleic acid sequence of the ORF of glycoprotein GP, the matrix protein Z, the nucleoprotein NP, or the polymerase protein L. In other examples, the nucleic acid sequence encoding the HBV antigen is fused to the ORF of glycoprotein GP, the matrix protein Z, the nucleoprotein NP, or the polymerase protein L. The nucleotide sequence encoding the HBV antigen, once inserted into the genome of an infectious arenavirus, can be transcribed and/or expressed under control of one of the four arenavirus promoters (5' UTR and 3' UTR of the S segment, and 5' UTR and 3' UTR of the L segment), as well as ribonucleic acids that can be inserted with regulatory elements that can be read by the viral RNA-dependent RNA polymerase, cellular RNA polymerase I, RNA polymerase II or RNA polymerase III, such as duplications of viral promoter sequences that are naturally found in the viral UTRs, the 28S ribosomal RNA promoter, the beta-actin promoter or the 5S ribosomal RNA promoter, respectively. The nucleic acids encoding the HBV antigen can be transcribed and/or expressed either by themselves or as read-through by fusion to arenavirus ORFs and genes, respectively, and/or in combination with one or more, e.g., two, three or four, internal ribosome entry sites.

[0135] In one embodiment, the antigen is one that is useful for the prevention and/or treatment of infectious disease. In a specific embodiment, the antigen is derived from HBV. In certain embodiments, the ORF that encodes the glycoprotein of the arenavirus is substituted by a nucleic acid sequence encoding HBV pre-S2/S protein. In certain embodiments, the ORF that encodes the glycoprotein of the arenavirus is substituted by a nucleic acid sequence encoding HBV HBc protein.

[0136] In certain embodiments, the ORF that encodes the glycoprotein of the arenavirus is substituted by a nucleic acid sequence encoding a fusion of HBV HBs and HBc proteins or antigenic fragments thereof.

(g) Substitution of the ORF encoding the glycoprotein of the arenavirus

[0137] In certain embodiments, the ORF that encodes the glycoprotein of the arenavirus is substituted by a nucleic acid sequence encoding one, two, or more HBV antigens described herein.

[0138] In one embodiment, the ORF that encodes the glycoprotein of the arenavirus is substituted by nucleic acid sequences encoding an HBV antigen. In certain embodiments, the ORF that encodes the glycoprotein of the arenavirus is substituted by nucleic acid sequences encoding an antigen that is a fragment of at least 10, 15, 20, 25, 30, 35, 40, 45, 50, or more amino acids of a gene product of a gene of the pre-S2/S protein of HBV or a fragment thereof. In certain embodiments, the ORF that encodes the glycoprotein of the arenavirus is substituted by nucleic acid sequences encoding an antigenic fragment of pre-S2/S. In certain embodiments, the ORF that encodes the glycoprotein of the arenavirus is substituted by nucleic acid sequences encoding antigens including, but not limited to pre-S2/S or a fragment of pre-S2/S.

[0139] In certain embodiments, the ORF that encodes the glycoprotein of the arenavirus is substituted by nucleic acid sequences encoding an antigen that is a fragment of at least 10, 15, 20, 25, 50, 75, 100, 125, 150 or more amino acids of a gene product of a gene of the HBc protein of HBV or a fragment thereof. In certain embodiments, the ORF that encodes the glycoprotein of the arenavirus is substituted by nucleic acid sequences encoding an antigenic fragment of HBc. In certain embodiments, the ORF that encodes the glycoprotein of the arenavirus is substituted by nucleic acid sequences encoding antigens including, but not limited to HBc or a fragment of HBc.

[0140] In certain examples, not in the context of the invention, the ORF that encodes the glycoprotein of the arenavirus is substituted by nucleic acid sequences encoding an antigen that is a fragment of at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or more amino acids of a gene product of a gene of the HBs protein of HBV or a fragment thereof. In certain examples, not in the context of the invention, the ORF that encodes the glycoprotein of the arenavirus is substituted by nucleic acid sequences encoding an antigenic fragment of HBs. In certain examples, not in the context of the invention, the ORF that encodes the glycoprotein of the arenavirus is substituted by nucleic acid sequences encoding antigens including, but not limited to HBs or a fragment of HBs.

[0141] In certain embodiments, the ORF that encodes the glycoprotein of the arenavirus is substituted by a nucleic acid sequence encoding two or more HBV proteins or fragments of at least 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225 or more amino acids thereof. In certain embodiments, the ORF that encodes the glycoprotein of the arenavirus is substituted by a nucleic acid sequence encoding HBs and HBc.

[0142] In certain embodiments, the ORF that encodes the glycoprotein of the arenavirus is substituted by a nucleic acid sequence encoding one or more of pre-S2/S protein or an antigenic fragment thereof, HBc protein or an antigenic fragment thereof,

6.3 Generation of Infectious Arenavirus Expressing an HBV Antigen

[0143] Generally, arenavirus particles can be recombinantly produced by standard reverse genetic techniques as described for LCMV (L. Flatz, A. Bergthaler, J. C. de la Torre, and D. D. Pinschewer, Proc Natl Acad Sci USA 103:4663-4668, 2006; A. B. Sanchez and J. C. de la Torre, Virology 350:370, 2006; E. Ortiz-Riano, B.Y. Cheng, J. C. de la Torre, L. Martinez-Sobrido. J Gen Virol. 94:1175-88, 2013).

(a) Replication-deficient arenaviruses

[0144] To generate infectious, replication-deficient arenaviruses for use with the present invention these techniques can be used, however, the genome of the rescued virus is modified as described in Section 6.1. These modifications can be: i) one or more, e.g., two, three or four, of the four arenavirus ORFs (glycoprotein (GP); nucleoprotein (NP); the matrix protein Z; the RNA-dependent RNA polymerase L) are removed or functionally inactivated to prevent formation of infectious particles in normal cells albeit still allowing gene expression in arenavirus vector-infected host cells; and ii) nucleic acids coding for HBV antigens can be introduced. Infectious, replication-deficient viruses as described herein can be produced as described in International Patent Application Publication No. WO 2009/083210 (application number PCT/EP2008/010994) and International Patent Application Publication No. WO 2014/140301 (application number PCT/EP2014/055144),

[0145] Once generated from cDNA, the infectious, replication-deficient arenaviruses provided herein can be propagated in complementing cells. Complementing cells are cells that provide the functionality that has been eliminated from the replication-deficient arenavirus by modification of its genome (e.g., if the ORF encoding the GP protein is deleted or functionally inactivated, a complementing cell does provide the GP protein).

[0146] Owing to the removal or functional inactivation of one or more of the viral genes in arenavirus vectors (here deletion of the glycoprotein, GP, will be taken as an example), arenavirus vectors can be generated and expanded in cells providing in trans the deleted viral gene(s), e.g., the GP in the present example. Such a complementing cell line,

henceforth referred to as C-cells, is generated by transfecting a mammalian cell line such as BHK-21, HEK 293, VERO or other (here BHK-21 will be taken as an example) with one or more plasmid(s) for expression of the viral gene(s) of interest (complementation plasmid, referred to as C-plasmid). The C-plasmid(s) express the viral gene(s) deleted in the arenavirus vector to be generated under control of one or more expression cassettes suitable for expression in mammalian cells, e.g., a mammalian polymerase II promoter such as the CMV or EF1alpha promoter with a polyadenylation signal. In addition, the complementation plasmid features a mammalian selection marker, e.g., puromycin resistance, under control of an expression cassette suitable for gene expression in mammalian cells, e.g., polymerase II expression cassette as above, or the viral gene transcript(s) are followed by an internal ribosome entry site, such as the one of encephalomyocarditis virus, followed by the mammalian resistance marker. For production in E. coli, the plasmid additionally features a bacterial selection marker, such as an ampicillin resistance cassette.

[0147] Cells that can be used, e.g., BHK-21, HEK 293, MC57G or other, are kept in culture and are transfected with the complementation plasmid(s) using any of the commonly used strategies such as calcium-phosphate, liposome-based protocols or electroporation. A few days later the suitable selection agent, e.g., puromycin, is added in titrated concentrations. Surviving clones are isolated and subcloned following standard procedures, and high-expressing C-cell clones are identified using Western blot or flow cytometry procedures with antibodies directed against the viral protein(s) of interest. As an alternative to the use of stably transfected C-cells transient transfection of normal cells can complement the missing viral gene(s) in each of the steps where C-cells will be used below. In addition, a helper virus can be used to provide the missing functionality in trans.

[0148] Plasmids that can be used can be of two types: i) Two plasmids, referred to as TF-plasmids for expressing intracellularly in C-cells the minimal transacting factors of the arenavirus, of which the replication-deficient arenavirus vector is derived from e.g., NP and L proteins of LCMV in the present example; and ii) Plasmids, referred to as GS-plasmids, for expressing intracellularly in C-cells the arenavirus vector genome segments, e.g., the segments with designed modifications. TF-plasmids express the NP and L proteins of the respective arenavirus vector under control of an expression cassette suitable for protein expression in mammalian cells, typically e.g., a mammalian polymerase II promoter such as the CMV or EF1 alpha promoter, either one of them preferentially in combination with a polyadenylation signal. GS-plasmids express the small (S) and the large (L) genome segments of the vector. Typically, polymerase I-driven

expression cassettes or T7 bacteriophage RNA polymerase (T7-) driven expression cassettes can be used, the latter preferentially with a 3'-terminal ribozyme for processing of the primary transcript to yield the correct end. In the case of using a T7-based system, expression of T7 in C-cells must be provided by either including in the recovery process an additional expression plasmid, constructed analogously to TF-plasmids, providing T7, or C-cells are constructed to additionally express T7 in a stable manner. In certain embodiments, TF and GS plasmids can be the same, i.e. the genome sequence and transacting factors can be transcribed by T7, polI and polII promoters from one plasmid.

[0149] For recovering of the arenavirus vector, the following procedures can be used. First day: C-cells, typically 80% confluent in M6-well plates, are transfected with a mixture of the two TF-plasmids plus the two GS-plasmids. In certain embodiments, the TF and GS plasmids can be the same, i.e. the genome sequence and transacting factors can be transcribed by T7, polI and polII promoters from one plasmid. For this one can exploit any of the commonly used strategies such as calcium-phosphate, liposome-based protocols or electroporation.

[0150] 3-5 days later: The culture supernatant (arenavirus vector preparation) is harvested, aliquoted and stored at 4°C, -20°C or -80°C depending on how long the arenavirus vector should be stored prior to use. Then the arenavirus vector preparation's infectious titer is assessed by an immunofocus assay on C-cells.

[0151] The invention furthermore relates to expression of an HBV antigen in a cell culture wherein the cell culture is infected with an infectious arenavirus expressing an HBV antigen. When used for expression of an HBV antigen in cultured cells, the following two procedures can be used:

i. The cell type of interest is infected with the arenavirus vector preparation described herein at a multiplicity of infection (MOI) of one or more, e.g., two, three or four, resulting in production of the HBV antigen in all cells already shortly after infection.

ii. Alternatively, a lower MOI can be used and individual cell clones can be selected for their level of virally driven HBV antigen expression. Subsequently individual clones can be expanded infinitely owing to the non-cytolytic nature of arenavirus vectors. Irrespective of the approach, the HBV antigen can subsequently be collected (and purified) either from the culture supernatant or from the cells themselves, depending on the properties of the HBV antigen produced. However, the invention is not limited to these two strategies, and other ways of driving

expression of HBV antigen using infectious, replication-deficient arenaviruses as vectors may be considered.

[0152] Alternatively, a rescue system consisting of three plasmids can be used: (1) the first plasmid expresses the protein NP by transcription via Polymerase II and subsequent translation in transfected cells; (2) the second plasmid gives rise to the (negative-stranded) L-Segment of the LCMV genome by transcription via Polymerase I as well as the L protein by transcription via Polymerase II from the same template in the opposite direction of the Polymerase I promoter; (3) the third plasmid gives rise to the S-segment of the LCMV genome (encoding the antigen coding sequence instead of the LCMV glycoprotein) via transcription by Polymerase I. 3µg of each plasmid is used for electroporation of C-cells, followed by seeding of cells in 6-well plates and incubation at 37°C. After incubation, cells and supernatant from transfections are combined with freshly seeded C-cells, and vectors are harvested and cleared from cells & debris at a defined timepoint post infection. Once the vector has been generated, a nucleic acid encoding an antigen of an oncogenic virus and/or an immunomodulatory peptide, polypeptide, or protein (see Section 6.2) can be inserted into a plasmid from which a genomic segment of an infectious replication-deficient vector is transcribed by any technique known to the skilled artisan.

[0153] Owing to the removal or functional inactivation of one or more of the viral genes in arenavirus vectors (here deletion of the glycoprotein, GP, will be taken as an example) arenavirus vectors can be generated and expanded in cells that provide the deleted or functionally inactivated viral gene(s) (*e.g.*, the GP) *in trans*. The resulting virus itself is infectious but is unable to produce further infectious progeny particles in non-complementing cells due to the lack of the deleted or functionally inactivated viral gene(s) (*e.g.*, the GP). The complementing cell can provide the missing functionality either by stable transfection, transient transfection, or by infection with a helper virus that expresses the missing functionality.

[0154] In certain embodiments, the complementing cell provides the viral gene that has been deleted or functionally inactivated from the arenavirus vector genome. In a specific embodiment, the complementing cell provides the viral gene from a viral strain that is the same as the viral strain that was used to generate the genome of the arenavirus vector. In another embodiment, the complementing cell provides the viral gene from a viral strain that is different from the viral strain that was used to generate the genome of the arenavirus vector. For example, the viral gene provided in the complementing cell is obtained from the MP strain of LCMV and encodes a protein having the amino acid

sequence of SEQ ID NO: 15, 16, 17, or 18. In another example, the viral gene provided in the complementing cell is obtained from the Clone 13 strain of LCMV and encodes a protein having the amino acid sequence of SEQ ID NO: 21, 22, 23, or 24. In another example, the viral gene provided in the complementing cell is obtained from the WE strain of LCMV and encodes a protein having the amino acid sequence of SEQ ID NO: 25.

[0155] In a specific embodiment, the complementing cell provides the GP of the MP strain of LCMV and the arenavirus vector comprises an ORF of a human HBV antigen as described herein in place of the ORF encoding the GP protein. In an even more specific embodiment, the complementing cell provides the GP of the MP strain of LCMV and the arenavirus vector is obtained from LCMV Clone 13 and comprises an ORF of a human HBV antigen as described herein in place of the ORF encoding the GP protein. In an even more specific embodiment, the GP protein is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 16.

[0156] In a specific embodiment, the complementing cell provides the GP of the Clone 13 strain of LCMV and the arenavirus vector comprises an ORF of a human HBV antigen as described herein in place of the ORF encoding the GP protein. In an even more specific embodiment, the complementing cell provides the GP of the Clone 13 strain of LCMV and the arenavirus vector is obtained from LCMV MP strain and comprises an ORF of a human HBV antigen as described herein in place of the ORF encoding the GP protein. In an even more specific embodiment, the GP protein is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 22.

[0157] In a specific embodiment, the complementing cell provides the GP of the WE strain of LCMV and the arenavirus vector comprises an ORF of a human HBV antigen as described herein in place of the ORF encoding the GP protein. In an even more specific embodiment, the complementing cell provides the GP of the WE strain of LCMV and the arenavirus vector is obtained from LCMV Clone 13 and comprises an ORF of a human HBV antigen as described herein in place of the ORF encoding the GP protein. In an even more specific embodiment, the GP protein is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 25.

[0158] In a specific embodiment, the complementing cell provides the GP of the WE strain of LCMV and the arenavirus vector comprises an ORF of a human HBV antigen as described herein in place of the ORF encoding the GP protein. In an even more specific

embodiment, the complementing cell provides the GP of the WE strain of LCMV and the arenavirus vector is obtained from LCMV MP strain and comprises an ORF of a human HBV antigen as described herein in place of the ORF encoding the GP protein. In an even more specific embodiment, the GP protein is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 25.

[0159] In certain embodiments, the infectious, replication-deficient arenavirus is trisegmented.

(b) Replication-competent, trisegmented arenaviruses

[0160] Provided herein are in vitro methods of generation of replication-competent arenavirus vectors. Infectious, replication-competent trisegmented viruses as described herein can be produced as described in United States Provisional Patent Application No. 62/079,493.

[0161] In certain embodiments, the method of generating a tri-segmented arenavirus particle comprises (i) transfecting into a host cell the cDNAs of the one L segment and two S segments or two L segments and one S segment; (ii) transfecting into a host cell plasmids expressing the arenavirus' minimal trans-acting factors NP and L; (iii) maintaining the host cell under conditions suitable for virus formation; and (iv) harvesting the arenavirus particle.

[0162] Once generated from cDNA, the tri-segmented arenavirus particle (i.e., infectious and replication competent) can be propagated. In certain embodiments tri-segmented arenavirus particles can be propagated in any host cell that allows the virus to grow to titers that permit the uses of the virus as described herein. In one embodiment, the host cell allows the tri-segmented arenavirus particle to grow to titers comparable to those determined for the corresponding wild-type.

[0163] In certain embodiments, the tri-segmented arenavirus particle may be propagated in host cells. Specific examples of host cells that can be used include BHK-21, HEK 293, VERO or other. In a specific embodiment, the tri-segmented arenavirus particle may be propagated in a cell line.

[0164] In certain embodiments, the host cells are kept in culture and are transfected with one or more plasmid(s). The plasmid(s) express the arenavirus genomic segment(s) to be generated under control of one or more expression cassettes suitable for expression in mammalian cells, e.g., consisting of a polymerase I promoter and terminator.

[0165] In specific embodiments, the host cells are kept in culture and are transfected with one or more plasmid(s). The plasmid(s) express the viral gene(s) to be generated under control of one or more expression cassettes suitable for expression in mammalian cells, e.g., consisting of a polymerase I promoter and terminator.

[0166] Plasmids that can be used for generating a tri-segmented arenavirus comprising one L segment and two S segments can include: i) two plasmids each encoding the S genome segment e.g., pol-I driven S segment expression plasmids, ii) a plasmid encoding the L genome segment e.g., a pol-I driven L segment expression plasmid. Plasmids needed for the tri-segmented arenavirus comprising two L segments and one S segments are: i) two plasmids each encoding the L genome segment e.g., pol-L, ii) a plasmid encoding the S genome segment e.g., pol-I S.

[0167] In certain embodiments, plasmids encoding an arenavirus polymerase that direct intracellular synthesis of the viral L and S segments can be incorporated into the transfection mixture. For example, a plasmid encoding the L protein and a plasmid encoding NP (pC-L and pC-NP, respectively). The L protein and NP are the minimal trans-acting factors necessary for viral RNA transcription and replication. Alternatively, intracellular synthesis of viral L and S segments, together with NP and L protein can be performed using an expression cassette with pol-I and pol-II promoters reading from opposite sides into the L and S segment cDNAs of two separate plasmids, respectively.

[0168] In addition, the plasmid(s) features a mammalian selection marker, e.g., puromycin resistance, under control of an expression cassette suitable for gene expression in mammalian cells, e.g., polymerase II expression cassette as above, or the viral gene transcript(s) are followed by an internal ribosome entry site, such as the one of encephalomyocarditis virus, followed by the mammalian resistance marker. For production in E.coli, the plasmid additionally features a bacterial selection marker, such as an ampicillin resistance cassette.

[0169] Transfection of BHK-21 cells with a plasmid(s) can be performed using any of the commonly used strategies such as calcium-phosphate, liposome-based protocols or electroporation. A few days later the suitable selection agent, e.g., puromycin, is added in titrated concentrations. Surviving clones are isolated and subcloned following standard procedures, and high-expressing clones are identified using Western blot or flow cytometry procedures with antibodies directed against the viral protein(s) of interest.

[0170] Typically, RNA polymerase I-driven expression cassettes, RNA polymerase II-driven cassettes or T7 bacteriophage RNA polymerase driven cassettes can be used, the latter preferentially with a 3'-terminal ribozyme for processing of the primary transcript to

yield the correct end. In certain embodiments, the plasmids encoding the arenavirus genomic segments can be the same, i.e., the genome sequence and transacting factors can be transcribed by T7, polI and polII promoters from one plasmid.

[0171] For recovering the tri-segmented arenavirus vector, the following procedures are envisaged. First day: cells, typically 80% confluent in M6-well plates, are transfected with a mixture of the plasmids, as described above. For this one can exploit any commonly used strategies such as calcium-phosphate, liposome-based protocols or electroporation.

[0172] 3-5 days later: The cultured supernatant (arenavirus vector preparation) is harvested, aliquoted and stored at 4°C, -20°C, or -80°C, depending on how long the arenavirus vector should be stored prior use. The arenavirus vector preparation's infectious titer is assessed by an immunofocus assay. Alternatively, the transfected cells and supernatant may be passaged to a larger vessel (e.g., a T75 tissue culture flask) on day 3-5 after transfection, and culture supernatant is harvested up to five days after passage.

[0173] The present application furthermore relates to expression of a heterologous ORF (*e.g.,* an HBV antigen), wherein a plasmid encoding the genomic segment is modified to incorporate a heterologous ORF. The heterologous ORF can be incorporated into the plasmid using restriction enzymes. In certain embodiments, the heterologous ORF encodes an HBV antigen. In certain embodiments, the plasmid encoding the genomic segment is modified to incorporate one or more heterologous ORFs. In certain embodiments, the heterologous ORFs encode one or more HBV antigens.

6.4 Nucleic Acids, Vector Systems and Cell Lines

[0174] In one embodiment, described herein is a nucleic acid sequence which is the cDNA of the large genomic segment (L segment) of an infectious arenavirus described herein, in which one ORF of the genomic segment is deleted or functionally inactivated, and the genomic segment comprises a nucleotide sequence encoding an HBV antigen. In certain embodiments, the infectious arenavirus viral vector is replication-deficient (See Section 6.1(a)). In certain embodiments, the infectious arenavirus viral vector is replication-competent (See Section 6.1(b)).

[0175] In one embodiment, described herein is a nucleic acid sequence that encodes the short genomic segment (S segment) of an infectious arenavirus described herein, in which one ORF of the genomic segment is deleted or functionally inactivated and wherein the short genomic segment comprises a nucleotide sequence encoding an HBV antigen.

In another embodiment, described herein is a nucleic acid sequence that encodes the short genomic segment (S segment) of an infectious arenavirus described herein, in which the ORF of the glycoprotein gene is deleted or functionally inactivated and wherein the short genomic segment comprises a nucleotide sequence encoding an HBV antigen. In certain, more specific embodiments, the HBV antigen is an antigen described in Section 6.2.

[0176] In certain embodiments, the nucleic acid sequences provided herein can be derived from a particular strain of LCMV. Strains of LCMV include Clone 13, MP strain, Arm CA 1371, Arm E-250, WE, UBC, Traub, Pasteur, 810885, CH-5692, Marseille #12, HP65-2009, 200501927, 810362, 811316, 810316, 810366, 20112714, Douglas, GR01, SN05, CABN and their derivatives. In specific embodiments, the nucleic acid is derived from LCMV Clone 13. In other specific embodiments, the nucleic acid is derived from LCMV MP strain.

[0177] In a more specific embodiment, provided herein is a nucleic acid comprising an arenavirus genomic segment comprising a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, at least 99%, or 100% identical to the sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In another embodiment, provided herein is a nucleic acid that comprises an arenavirus genomic segment comprising (i) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, at least 99%, or 100% identical to the sequence of nucleotide 1639 to 3315 of SEQ ID NO: 11; and (ii) a nucleotide sequence encoding an HBV antigen.

[0178] In another embodiment, provided herein is a nucleic acid that comprises an arenavirus genomic segment comprising (i) a nucleotide sequence encoding an expression product whose amino acid sequence is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, at least 99%, or 100% identical to the amino acid sequence encoded by 1639 to 3315 of SEQ ID NO: 11; and (ii) a nucleotide sequence encoding an HBV antigen.

[0179] In another embodiment, provided herein is a nucleic acid that comprises an arenavirus genomic segment comprising (i) a nucleotide sequence that is at least 90%, 91%), 92%, 93%, 94%, 95%, 96%, 97%, 98%, at least 99%, or 100% identical to the sequence of nucleotide 1640 to 3316 of SEQ ID NO: 12; and (ii) a nucleotide sequence encoding an HBV antigen.

[0180] In another embodiment, provided herein is a nucleic acid that comprises an arenavirus genomic segment comprising (i) a nucleotide sequence encoding an expression product whose amino acid sequence is at least 90%, 91%, 92%, 93%, 94%,

95%, 96%, 97%, 98%, at least 99%, or 100% identical to the amino acid sequence encoded by 1640 to 3316 of SEQ ID NO: 12; and (ii) a nucleotide sequence encoding an HBV antigen

[0181] In one embodiment, described herein is a vector system comprising one or more vectors that together comprise the genome of an infectious arenavirus particle described herein. Specifically, provided herein is a vector system wherein the one or more vectors comprise two arenavirus genomic segments, namely an L segment and an S segment, of an infectious arenavirus described herein. Such a vector system can comprise (on one or more separate DNA molecules):

[0182] An arenavirus S genomic segment that is modified such that an arenavirus particle carrying this modified S genomic segment cannot produce infectious progeny virus particles and an arenavirus L genomic segment that comprises a nucleotide sequence encoding (in sense or antisense) an HBV antigen;

[0183] An arenavirus L genomic segment that is modified such that an arenavirus particle carrying this modified L genomic segment cannot produce infectious progeny virus particles and an arenavirus S genomic segment that comprises a nucleotide sequence encoding (in sense or antisense) an HBV antigen;

[0184] An arenavirus S genomic segment that is modified such that an arenavirus particle carrying this modified S genomic segment cannot produce infectious progeny virus particles and wherein the arenavirus S genomic segment comprises a nucleotide sequence encoding (in sense or antisense) an HBV antigen and comprising a wild type arenavirus L genomic segment; or

[0185] An arenavirus L genomic segment that is modified such that an arenavirus particle carrying this modified L genomic segment cannot produce infectious progeny virus particles and wherein the arenavirus L genomic segment comprises a nucleotide sequence encoding (in sense or antisense) an HBV antigen and comprising a wild type arenavirus S genomic segment.

[0186] In the context of the invention, provided herein is a nucleic acid sequence comprising an arenavirus (e.g., LCMV) genomic segment in which the ORF encoding the GP of the S genomic segment is substituted with a nucleotide sequence comprising:

a nucleotide sequence encoding a Hepatitis B pre-S2/S protein or an antigenic fragment thereof;

a nucleotide sequence encoding a Hepatitis B virus HBc protein or an antigenic fragment thereof; or

a nucleotide sequence encoding a fusion of Hepatitis B virus HBs and HBc proteins or antigenic fragments thereof;

and wherein a viral vector comprising the arenavirus genomic segment is capable of eliciting a T. cell response against the HBV pre-S2/S protein, the HBc protein, the fusion of HBs and HBc proteins, or an antigenic fragment thereof .

[0187] In certain embodiments, provided herein is a nucleic acid sequence comprising an arenavirus (e.g., LCMV) genomic segment in which the ORF encoding the GP of the S genomic segment is substituted with a nucleotide sequence encoding one or more HBV antigens (e.g., one or more of those listed in the above paragraph).

[0188] In another embodiment, a cell may comprise a nucleic acid or a vector system described above in this section. Cell lines derived from such cells, cultures comprising such cells, and methods of culturing such cells infected with nucleic acids or vector systems are also described. In certain embodiments, the cell may comprise a nucleic acid comprising the large genomic segment (L segment) of an infectious arenavirus described herein, in which one ORF of the genomic segment is deleted or functionally inactivated, and the genomic segment comprises a nucleotide sequence encoding an HBV antigen.

[0189] In other embodiments, the cell may comprise a nucleic acid sequence that comprises the short genomic segment (S segment) of an infectious arenavirus provided herein, in which one ORF of the genomic segment is deleted or functionally inactivated and wherein the short genomic segment comprises a nucleotide sequence encoding HBV pre-S2/S protein or an antigenic fragment thereof.

[0190] In other embodiments, a cell may comprise a nucleic acid sequence that comprises the short genomic segment (S segment) of an infectious arenavirus provided herein, in which one ORF of the genomic segment is deleted or functionally inactivated and wherein the short genomic segment comprises a nucleotide sequence encoding HBV HBc protein or an antigenic fragment thereof.

[0191] In examples, not in the context of the invention, a cell may comprise a nucleic acid sequence that comprises the short genomic segment (S segment) of an infectious arenavirus, in which one ORF of the genomic segment is deleted or functionally inactivated and wherein the short genomic segment comprises a nucleotide sequence encoding HBV HBs protein or an antigenic fragment thereof.

[0192] In other embodiments, a cell may comprise a nucleic acid sequence that comprises the short genomic segment (S segment) of an infectious arenavirus provided herein, in which one ORF of the genomic segment is deleted or functionally inactivated and wherein the short genomic segment comprises a nucleotide sequence encoding a

fusion protein comprising at least one domain from HBV HBs protein and HBV HBc protein.

[0193] In other embodiments, a cell may comprise a nucleic acid sequence that comprises the short genomic segment (S segment) of an infectious arenavirus provided herein, in which one ORF of the genomic segment is deleted or functionally inactivated and wherein the short genomic segment comprises a nucleotide sequence encoding one or more of HBV antigens.

[0194] In another embodiment, a cell may comprise two nucleic acids or vector systems described herein.

[0195] In certain embodiments, a nucleic acid comprises a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 13 or SEQ ID NO: 14. In certain embodiments, an expression vector comprises a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 13 or SEQ ID NO: 14. In certain embodiments, a host cell comprises a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 13 or SEQ ID NO: 14.

[0196] In certain embodiments, a nucleic acid comprises a nucleotide sequence encoding an amino acid sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 15, 16, 17, or 18. In certain embodiments, an expression vector comprises a nucleotide sequence encoding an amino acid sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 15, 16, 17, or 18. In certain embodiments, a host cell comprises a nucleotide sequence that encodes an amino acid sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 15, 16, 17, or 18.

[0197] In certain embodiments, an isolated protein comprises an amino acid sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 15, 16, 17, or 18. In certain embodiments, a host cell expresses a protein comprising an amino acid sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%,

93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 15, 16, 17, or 18. In certain embodiments, the host cell is cultured in cell culture medium.

[0198] In certain embodiments, a nucleic acid comprises a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 12 or SEQ ID NO: 7. In certain embodiments, an expression vector comprises a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 12 or SEQ ID NO: 7. In certain embodiments, a host cell comprises a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 12 or SEQ ID NO: 7.

[0199] In certain embodiments, a nucleic acid comprises a nucleotide sequence encoding an amino acid sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 21, 22, 23, or 24. In certain embodiments, an expression vector comprises a nucleotide sequence encoding an amino acid sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 21, 22, 23, or 24. In certain embodiments, a host cell comprises a nucleotide sequence that encodes an amino acid sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 21, 22, 23, or 24.

[0200] In certain embodiments, an isolated protein comprises an amino acid sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 21, 22, 23, or 24. In certain embodiments, a host cell expresses a protein comprising an amino acid sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 21, 22, 23, or 24. In certain embodiments, the host cell is cultured in cell culture medium.

6.5 Methods of Use

[0201] Provided herein are arenavirus viral vectors according to the invention, for use in immunotherapies for Hepatitis B virus infections. In one embodiment, provided herein are one or more infectious arenaviruses expressing an HBV antigen as provided herein or a composition thereof for use in methods of treating an infection in a subject comprising

administering to the subject one or more infectious arenaviruses expressing an HBV antigen as provided herein or a composition thereof. In certain embodiments, the infectious arenaviruses are replication-deficient. In certain embodiments, the infectious arenaviruses are replication-competent. In a specific embodiment, a method for treating an infection comprises administering to a subject in need thereof an effective amount of one or more infectious arenaviruses expressing an HBV antigen provided herein or a composition thereof. The subject can be a mammal, such as but not limited to a human being, a mouse, a rat, a guinea pig, a domesticated animal, such as, but not limited to, a cow, a horse, a sheep, a pig, a goat, a cat, a dog, a hamster, a donkey. In a specific embodiment, the subject is a human.

[0202] In another embodiment, methods for inducing an immune response against HBV in a subject comprise administering to the subject an infectious arenavirus expressing an HBV antigen or a composition thereof.

[0203] In another embodiment, the subjects to whom an infectious arenavirus expressing an HBV antigen described herein or a composition thereof is administered have, are susceptible to, or are at risk for an HBV infection. In another specific embodiment, the subjects to whom an infectious arenavirus expressing an HBV antigen described herein or a composition thereof is administered are infected with, are susceptible to, or are at risk for, an infection with HBV.

[0204] In another embodiment, the subjects to whom an infectious arenavirus expressing an HBV antigen described herein or a composition thereof is administered are suffering from, are susceptible to, or are at risk for, an infection with HBV, *e.g.,* in the liver. In a specific embodiment, the subjects to whom an infectious arenavirus expressing an HBV antigen described herein or a composition thereof is administered are suffering from, are susceptible to, or are at risk for, an infection with HBV in one or more organs of the body, e.g., the liver.

[0205] In another embodiment, the subjects to whom an infectious arenavirus expressing an HBV antigen described herein or a composition thereof is administered have test results (e.g., blood test results) indicating liver damage. In certain embodiments, the subjects have alanine aminotransferase (ALT) levels in the blood indicating liver damage. In certain embodiment, the subjects have aspartate aminotransferase (AST) levels in the blood indicating liver damage. In certain embodiments, the subjects have alkaline phosphatase levels in the blood indicating liver damage. In certain embodiments, the subjects have lactate dehydrogenase (LDH) levels in the blood indicating liver damage.

In certain embodiments, the subjects have one or more of ALT, AST, alkaline phosphatase, and LDH levels in the blood indicating liver damage.

[0206] In certain embodiments, the subjects have alpha-fetoprotein (AFP) levels in the blood indicating liver cancer or susceptibility thereto. In certain embodiments, the subjects have bilirubin (e.g., conjugated bilirubin) levels in the blood indicating liver damage. In certain embodiments, the subjects have albumin levels in the blood indicating liver damage.

[0207] In certain embodiments, the subjects have abdominal ultrasound results indicating liver damage. In certain embodiments, the subjects have CAT scan results indicating liver damage. In certain embodiments, the subjects have MRI results indicating liver damage.

[0208] In another embodiment, the subjects to whom an infectious arenavirus expressing an HBV antigen described herein or a composition thereof is administered have detectable levels of HBs antigen (HBsAg) in the blood. In certain embodiments, the subjects have detectable levels of IgM antibody against HBc antigen (HBcAg) in the blood. In certain embodiments, the subjects have detectable levels of HBe antigen (HBeAg, the extracellular/secreted version of the HBc protein) in the blood. In certain embodiments, the subjects have detectable levels of antibody to HBsAg in the blood.

[0209] In another embodiment, the subjects to whom an infectious arenavirus expressing an HBV antigen described herein or a composition thereof is administered have persistent levels of HBsAg, indicative of chronic hepatitis. In certain embodiments, the subjects have persistent levels of HBeAg, indicative of chronic hepatitis. In certain embodiments, the subjects have persistent levels of HBsAg and HBeAg, indicative of chronic hepatitis.

[0210] In another embodiment, the subjects to whom an infectious arenavirus expressing an HBV antigen described herein or a composition thereof is administered are suffering from symptoms of HBV infection, including but not limited to loss of appetite, fatigue, nausea, vomiting, itchiness, abdominal pain, abdominal swelling, or jaundice.

[0211] In another embodiment, the subjects to whom an infectious arenavirus expressing an HBV antigen described herein or a composition thereof is administered are suffering from manifestations of HBV, including but not limited to acute hepatitis B, chronic HBV infection, cirrhosis, and hepatocellular carcinoma (HCC). In another embodiment, the infectious arenavirus expressing an HBV antigen described herein or a composition thereof is administered to a subject with asymptomatic HBV.

[0212] In another embodiment, an infectious arenavirus expressing an HBV antigen as described herein or a composition thereof is administered to a subject of any age group suffering from, susceptible to, or at risk for, an infection with HBV. In a specific

embodiment, an infectious arenavirus expressing an HBV antigen as described herein or a composition thereof is administered to a subject with a compromised immune system, a pregnant subject, a subject undergoing an organ or bone marrow transplant, a subject taking immunosuppressive drugs, a subject undergoing hemodialysis, a subject who has cancer, or a subject who is suffering from, susceptible to, or at risk for, an infection with HBV. In a more specific embodiment, an infectious arenavirus expressing an HBV antigen as described herein or a composition thereof is administered to a subject with a compromised immune system due to HIV infection, who is suffering from, is susceptible to, or is at risk for, an infection with HBV. In yet another specific embodiment, an infectious arenavirus expressing an HBV antigen as described herein or a composition thereof is administered to a subject who is a child of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 years of age suffering from, susceptible to, or at risk for, an infection with HBV. In yet another specific embodiment, an infectious arenavirus expressing an HBV antigen described herein or a composition thereof is administered to a subject who is an infant suffering from, susceptible to, or at risk for, an infection with HBV. In yet another specific embodiment, an infectious arenavirus expressing an HBV antigen described herein or a composition thereof is administered to a subject who is an infant of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months of age suffering from, susceptible to, or at risk for, an infection with HBV. In yet another specific embodiment, an infectious arenavirus expressing an HBV antigen described herein or a composition thereof is administered to an elderly subject who is suffering from, is susceptible to, or is at risk for, an infection with HBV.

[0213] In another embodiment, an infectious arenavirus expressing an HBV antigen described herein or a composition thereof is administered to subjects with a heightened risk of disseminated HBV infection. In a specific embodiment, an infectious arenavirus expressing an HBV antigen described herein or a composition thereof is administered to subjects in neonatal period with immature neonatal immune system. In another embodiment, an infectious arenavirus expressing an HBV antigen described herein or a composition thereof is administered to a subject who uses intravenous drugs with a heightened risk of HBV infection.

[0214] In another embodiment, an infectious arenavirus expressing an HBV antigen described herein or a composition thereof is administered to subjects infected with one or more genotypes or subgenotypes of HBV. In certain embodiments, the genotype is one or more of genotypes A-J, or another genotype. In certain embodiments, the

subgenotype is one or more subgenotypes A1-A6, B1-B4, C1-C6, D1-D7, F1-F4, or another subgenotype.

[0215] In another embodiment, administering an infectious arenavirus expressing an HBV antigen as described herein or a composition thereof to subjects confer cell-mediated immunity (CMI) against an infection with HBV. Without being bound by theory, in another embodiment, an infectious arenavirus expressing an HBV antigen as described herein or a composition thereof infects and expresses antigens of interest in antigen presenting cells (APC) of the host (e.g., macrophages) for direct presentation of antigens on Major Histocompatibility Complex (MHC) class I and II. In another embodiment, administering an infectious arenavirus expressing an HBV antigen as described herein or a composition thereof to subjects induces plurifunctional IFN-γ and TNF-α co-producing HBV-specific CD4+ and CD8+ T cell responses (IFN-γ is produced by CD4+ and CD8+ T cells and TNF-α is produced by CD4+ T cells) of high magnitude to treat or prevent an infection with HBV.

[0216] In another embodiment, administering an infectious arenavirus expressing an HBV antigen or a composition thereof reduces the risk that an individual will develop an infection with HBV by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the risk of developing an infection with HBV in the absence of such treatment.

[0217] In another embodiment, administering an infectious arenavirus expressing an HBV antigen or a composition thereof reduces the symptoms of an infection with HBV by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the manifestation of the symptoms of an infection with HBV in the absence of such treatment.

[0218] In another embodiment, administering an infectious arenavirus expressing an HBV antigen or a composition thereof to subjects with immature neonatal immune system induces cell-mediated immunity (CMI) response against an infection with HBV by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to cell-mediated immunity (CMI) response against an infection with HBV in the absence of such a treatment.

[0219] In certain embodiments, administering an infectious arenavirus expressing an HBV antigen or a composition thereof reduces ALT levels in the blood. In certain

embodiments, administering an infectious arenavirus expressing an HBV antigen or a composition thereof reduces AST levels in the blood. In certain embodiments, administering an infectious arenavirus expressing an HBV antigen or a composition thereof reduces alkaline phosphatase levels in the blood. In certain embodiments, administering an infectious arenavirus expressing an HBV antigen or a composition thereof reduces LDH levels in the blood. In certain embodiments, administering an infectious arenavirus expressing an HBV antigen or a composition thereof reduces one or more of ALT, AST, alkaline phosphatase, and LDH levels in the blood.

[0220] In certain embodiments, administering an infectious arenavirus expressing an HBV antigen or a composition thereof reduces AFP levels in the blood. In certain embodiments, administering an infectious arenavirus expressing an HBV antigen or a composition thereof reduces bilirubin (e.g., conjugated bilirubin) levels in the blood. In certain embodiments, administering an infectious arenavirus expressing an HBV antigen or a composition thereof increases albumin levels in the blood.

[0221] In certain embodiments, administering an infectious arenavirus expressing an HBV antigen or a composition thereof reduces levels of HBsAg in the blood. In certain embodiments, administering an infectious arenavirus expressing an HBV antigen or a composition thereof reduces levels of IgM antibody against HBcAg in the blood. In certain embodiments, administering an infectious arenavirus expressing an HBV antigen or a composition thereof reduces levels of HBeAg in the blood. In certain embodiments, administering an infectious arenavirus expressing an HBV antigen or a composition thereof reduces levels of antibody to HBsAg in the blood.

[0222] In certain embodiments, administering an infectious arenavirus expressing an HBV antigen or a composition thereof reduces the number of inclusion bodies detected in salivary glands or another histological sample. In certain embodiments, administering an infectious arenavirus expressing an HBV antigen or a composition thereof reduces the number of anti-HBV antibodies detected in a patient blood sample. In certain embodiments, administering an infectious arenavirus expressing an HBV antigen or a composition thereof reduces the amount of HBV detected in urine, saliva, blood, tears, semen, or breast milk. In certain embodiments, administering an infectious arenavirus expressing an HBV antigen or a composition thereof reduces the level of virus cultured from a urine, throat swab, bronchial lavage, or tissue sample. In certain embodiments, administering an infectious arenavirus expressing an HBV antigen or a composition thereof reduces the level of virus detected through quantitative or qualitative PCR tests.

[0223] Changes in cell-mediated immunity (CMI) response function against an infection with HBV induced by administering an infectious arenavirus expressing an HBV antigen or a composition thereof in subjects can be measured by any assay known to the skilled artisan including, but not limited to flow cytometry (see, e.g., Perfetto S.P. et al., Nat Rev Immun. 2004; 4(8):648-55), lymphocyte proliferation assays (see, e.g., Bonilla F.A. et al., Ann Allergy Asthma Immunol. 2008; 101:101-4; and Hicks M.J. et al., Am J Clin Pathol. 1983; 80:159-63), assays to measure lymphocyte activation including determining changes in surface marker expression following activation of measurement of cytokines of T lymphocytes (see, e.g., Caruso A. et al., Cytometry. 1997;27:71-6), ELISPOT assays (see, e.g., Czerkinsky C.C. et al., J Immunol Methods. 1983; 65:109-121; and Hutchings P.R. Et al., J Immunol Methods. 1989; 120:1-8), or Natural killer cell cytotoxicity assays (see, e.g., Bonilla F.A. et al., Ann Allergy Asthma Immunol. 2005 May; 94(5 Suppl 1):S1-63).

[0224] In another embodiment, described herein is a use of an infectious arenavirus (e.g., LCMV) expressing an HBV antigen as described herein in which the ORF encoding the GP of the S genomic segment is substituted with a nucleotide sequence comprising:

a. a nucleotide sequence encoding an HBV pre-S2/S protein or an antigenic fragment thereof;
b. a nucleotide sequence encoding an HBV HBc protein or an antigenic fragment thereof; or
c. a nucleotide sequence encoding a fusion of HBV HBs and HBc proteins or antigenic fragments thereof;

and, wherein the arenavirus is capable of eliciting a T cell response against the HBV pre-S2/S protein, the HBc protein, the fusion of HBs and HBc proteins, or an antigenic fragment thereof.

[0225] In another embodiment, provided herein is an infectious arenavirus expressing an HBV antigen as claimed for use in methods of preventing transmission and/or infection of HBV from a mother to an unborn child, the methods comprise administering to a subject of child-bearing age the infectious arenavirus expressing an HBV antigen. See Section 6.2. In specific embodiments, methods of preventing transmission and/or infection of HBV from a mother to an unborn child comprise administering to a seronegative subject of child-bearing age an infectious arenavirus expressing an HBV antigen as described herein. In yet another embodiment, methods of preventing transmission and/or infection of HBV from a mother to an unborn child comprise administering to a subject of child-

bearing age with the intention to procreate an infectious arenavirus expressing an HBV antigen as described herein.

[0226] In another embodiment, methods of preventing transmission and/or infection of HBV from a mother to an unborn child comprise administering to a subject of child-bearing age one or more infectious arenaviruses expressing an HBV antigen as described herein. See Section 6.2. In specific embodiments, methods of preventing transmission and/or infection of HBV from a mother to an unborn child comprise administering to a seronegative subject of child-bearing age one or more infectious arenaviruses expressing an HBV antigen as described herein. In yet another embodiment, methods of preventing transmission and/or infection of HBV from a mother to an unborn child comprise administering to a subject of child-bearing age with the intention to procreate one or more infectious arenaviruses expressing an HBV antigen as described herein.

[0227] In another embodiment, methods of preventing transmission and/or infection of HBV from a mother to an unborn child comprise administering to a pregnant subject an infectious arenavirus expressing an HBV antigen as described herein. In specific embodiments, methods of preventing transmission and/or infection of HBV from a mother to an unborn child comprise administering to a pregnant subject an effective amount of an infectious arenavirus expressing an HBV antigen described herein.

[0228] In another embodiment, methods of preventing transmission and/or infection of HBV from a mother to an unborn child comprise administering to a pregnant subject one or more infectious arenaviruses expressing an HBV antigen as described herein. In specific embodiments, methods of preventing transmission and/or infection of HBV from a mother to an unborn child comprise administering to a pregnant subject an effective amount of one or more infectious arenaviruses expressing an HBV antigen described herein.

[0229] In another embodiment, administering an infectious arenavirus expressing an HBV antigen reduces congenital HBV infection. In another embodiment, administering one or more infectious arenaviruses expressing an HBV antigen reduces congenital HBV infection.

[0230] In another embodiment, administering an infectious arenavirus expressing an HBV antigen reduces manifestations of congenital HBV infection by at least about 10%, at least about 20%, at least 25%, at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least 80%, at least 90%, or more. In another specific embodiment, administering an infectious arenavirus

expressing an HBV antigen reduces mortality of newborn infants with congenital HBV infection.

[0231] In another embodiment, administering one or more infectious arenaviruses expressing an HBV antigen reduces manifestations of congenital HBV infection by at least about 10%, at least about 20%, at least 25%, at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least 80%, at least 90%, or more. In another specific embodiment, administering one or more infectious arenaviruses expressing an HBV antigen reduces mortality of newborn infants with congenital HBV infection.

[0232] Such manifestations of congenital HBV include but are not limited to acute hepatitis B, chronic HBV infection, cirrhosis, and hepatocellular carcinoma (HCC).

6.6 Compositions, Administration and Dosage

[0233] The invention furthermore relates to vaccines, immunogenic compositions, and pharmaceutical compositions comprising a genetically engineered arenavirus as described herein. Such vaccines and pharmaceutical compositions can be formulated according to standard procedures in the art.

[0234] In another embodiment, provided herein are pharmaceutical compositions comprising an infectious arenavirus described herein. Such compositions can be used in methods of treatment and prevention of disease. In a specific embodiment, the compositions described herein are used in the methods of treatment of subjects infected with, or susceptible to, an infection with HBV. In another specific embodiment, the immunogenic compositions provided herein can be used to induce an immune response in a host to whom the composition is administered. The immunogenic compositions described herein can be used as vaccines and can accordingly be formulated as pharmaceutical compositions. In a specific embodiment, the immunogenic compositions provided herein are used in the methods of prevention of infection of subjects (e.g., human subjects) by HBV. In certain embodiments, the infectious arenavirus viral vector is replication-deficient (See Section 6.1(a)). In certain embodiments, the infectious arenavirus viral vector is replication-competent (See Section 6.1(b)).

[0235] In certain embodiments, provided herein are immunogenic compositions comprising an arenavirus vector (or a combination of different arenavirus vectors) as described herein. In certain embodiments, such an immunogenic composition further comprises a pharmaceutically acceptable excipient. In certain embodiments, such an

immunogenic composition further comprises an adjuvant. The adjuvant for administration in combination with a composition described herein may be administered before, concomitantly with, or after administration of said composition. In some embodiments, the term "adjuvant" refers to a compound that when administered in conjunction with or as part of a composition described herein augments, enhances and/or boosts the immune response to an infectious arenavirus particle, but when the compound is administered alone does not generate an immune response to the infectious arenavirus particle. In some embodiments, the adjuvant generates an immune response to the infectious arenavirus particle and does not produce an allergy or other adverse reaction. Adjuvants can enhance an immune response by several mechanisms including, e.g., lymphocyte recruitment, stimulation of B and/or T cells, and stimulation of macrophages. When a vaccine or immunogenic composition of the invention comprises adjuvants or is administered together with one or more adjuvants, the adjuvants that can be used include, but are not limited to, mineral salt adjuvants or mineral salt gel adjuvants, particulate adjuvants, microparticulate adjuvants, mucosal adjuvants, and immunostimulatory adjuvants. Examples of adjuvants include, but are not limited to, aluminum salts (alum) (such as aluminum hydroxide, aluminum phosphate, and aluminum sulfate), 3 De-O-acylated monophosphoryl lipid A (MPL) (see GB 2220211), MF59 (Novartis), AS03 (GlaxoSmithKline), AS04 (GlaxoSmithKline), polysorbate 80 (Tween 80; ICL Americas, Inc.), imidazopyridine compounds (see International Application No. PCT/US2007/064857, published as International Publication No. WO2007/109812), imidazoquinoxaline compounds (see International Application No. PCT/US2007/064858, published as International Publication No. WO2007/109813) and saponins, such as QS21 (see Kensil et al., in Vaccine Design: The Subunit and Adjuvant Approach (eds. Powell & Newman, Plenum Press, NY, 1995); U.S. Pat. No. 5,057,540). In some embodiments, the adjuvant is Freund's adjuvant (complete or incomplete). Other adjuvants are oil in water emulsions (such as squalene or peanut oil), optionally in combination with immune stimulants, such as monophosphoryl lipid A (see Stoute et al., N. Engl. J. Med. 336, 86-91 (1997)).

[0236] The compositions comprise the infectious arenaviruses described herein alone or together with a pharmaceutically acceptable carrier. Suspensions or dispersions of genetically engineered arenaviruses, especially isotonic aqueous suspensions or dispersions, can be used. The pharmaceutical compositions may be sterilized and/or may comprise excipients, e.g., preservatives, stabilizers, wetting agents and/or emulsifiers, solubilizers, salts for regulating osmotic pressure and/or buffers and are prepared in a

manner known per se, for example by means of conventional dispersing and suspending processes. In certain embodiments, such dispersions or suspensions may comprise viscosity-regulating agents. The suspensions or dispersions are kept at temperatures around 2-8°C, or preferentially for longer storage may be frozen and then thawed shortly before use. For injection, the vaccine or immunogenic preparations may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. The solution may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

[0237] In certain embodiments, the compositions described herein additionally comprise a preservative, e.g., the mercury derivative thimerosal. In a specific embodiment, the pharmaceutical compositions described herein comprise 0.001% to 0.01% thimerosal. In other embodiments, the pharmaceutical compositions described herein do not comprise a preservative.

[0238] The pharmaceutical compositions comprise from about 10^3 to about 10^{11} focus forming units of the genetically engineered arenaviruses. Unit dose forms for parenteral administration are, for example, ampoules or vials, e.g., vials containing from about 10^3 to 10^{10} focus forming units or 10^5 to 10^{15} physical particles of genetically engineered arenaviruses.

[0239] In another embodiment, a vaccine or immunogenic composition provided herein is administered to a subject by, including but not limited to, oral, intradermal, intramuscular, intraperitoneal, intravenous, topical, subcutaneous, percutaneous, intranasal and inhalation routes, and via scarification (scratching through the top layers of skin, e.g., using a bifurcated needle). Specifically, subcutaneous, intramuscular or intravenous routes can be used.

[0240] For administration intranasally or by inhalation, the preparation for use according to the present invention can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in an inhaler or insufflators may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0241] The dosage of the active ingredient depends upon the type of vaccination and upon the subject, and their age, weight, individual condition, the individual pharmacokinetic data, and the mode of administration.

[0242] Processes and uses of genetically engineered arenaviruses for the manufacture of vaccines in the form of pharmaceutical preparations, may comprise genetically engineered arenaviruses as active ingredient. The pharmaceutical compositions of the present invention are prepared in a manner known per se, for example by means of conventional mixing and/or dispersing processes.

6.7 Optimized Generation of LCMV Vectors

[0243] Owing to the removal or functional inactivation of one or more of the viral genes in arenavirus vectors (here deletion of the glycoprotein, GP, will be taken as an example) arenavirus vectors can be generated and expanded in cells that provide the deleted or functionally inactivated viral gene(s) (e.g., the GP) "in trans." The resulting virus itself is infectious but is unable to produce further infectious progeny particles in non-complementing cells due to the lack of the deleted or functionally inactivated viral gene(s) (e.g., the GP). The complementing cell can provide the missing functionality either by stable transfection, transient transfection, or by infection with a helper virus that expresses the missing functionality.

[0244] In certain embodiments, the complementing cell provides the viral gene that has been deleted or functionally inactivated from the arenavirus vector genome. In a specific embodiment, the complementing cell provides the viral gene from a viral strain that is the same as the viral strain that was used to generate the genome of the arenavirus vector. In another embodiment, the complementing cell provides the viral gene from a viral strain that is different from the viral strain that was used to generate the genome of the arenavirus vector. For example, the viral gene provided in the complementing cell is obtained from the MP strain of LCMV and encodes a protein having the amino acid sequence of SEQ ID NO: 15, 16, 17, or 18. In another example, the viral gene provided in the complementing cell is obtained from the Clone 13 strain of LCMV and encodes a protein having the amino acid sequence of SEQ ID NO: 21, 22, 23, or 24. In another example, the viral gene provided in the complementing cell is obtained from the WE strain of LCMV and encodes a protein having the amino acid sequence of SEQ ID NO: 25.

[0245] In a specific embodiment, the complementing cell provides the GP of the MP strain of LCMV and the arenavirus vector comprises an ORF of a human HBV antigen as described herein in place of the ORF encoding the GP protein. In an even more specific embodiment, the complementing cell provides the GP of the MP strain of LCMV and the arenavirus vector is obtained from LCMV Clone 13 and comprises an ORF of a human

HBV antigen as described herein in place of the ORF encoding the GP protein. In an even more specific embodiment, the GP protein is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 16.

[0246] In a specific embodiment, the complementing cell provides the GP of the Clone 13 strain of LCMV and the arenavirus vector comprises an ORF of a human HBV antigen as described herein in place of the ORF encoding the GP protein. In an even more specific embodiment, the complementing cell provides the GP of the Clone 13 strain of LCMV and the arenavirus vector is obtained from LCMV MP strain and comprises an ORF of a human HBV antigen as described herein in place of the ORF encoding the GP protein. In an even more specific embodiment, the GP protein is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 22.

[0247] In a specific embodiment, the complementing cell provides the GP of the WE strain of LCMV and the arenavirus vector comprises an ORF of a human HBV antigen as described herein in place of the ORF encoding the GP protein. In an even more specific embodiment, the complementing cell provides the GP of the WE strain of LCMV and the arenavirus vector is obtained from LCMV Clone 13 and comprises an ORF of a human HBV antigen as described herein in place of the ORF encoding the GP protein. In an even more specific embodiment, the GP protein is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 25.

[0248] In a specific embodiment, the complementing cell provides the GP of the WE strain of LCMV and the arenavirus vector comprises an ORF of a human HBV antigen as described herein in place of the ORF encoding the GP protein. In an even more specific embodiment, the complementing cell provides the GP of the WE strain of LCMV and the arenavirus vector is obtained from LCMV MP strain and comprises an ORF of a human HBV antigen as described herein in place of the ORF encoding the GP protein. In an even more specific embodiment, the GP protein is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 25.

6.8 Combination therapy

6.8 (a) Methods

[0249] In one embodiment, provided herein are two or more infectious arenaviruses as claimed, for use in methods of treating and/or preventing an HBV infection in a subject comprising administering to the subject said two or more infectious arenaviruses expressing an HBV antigen. See, e.g., Section 6.2. In specific embodiments, a method for treating and/or preventing an HBV infection comprises administering a first infectious arenavirus expressing an HBV antigen as described herein, e.g., in which the ORF encoding the GP of the S genomic segment is substituted with a nucleotide sequence encoding the HBV antigen, wherein the nucleotide sequence is selected from the group consisting of:

a) a nucleotide sequence encoding an HBV pre-S2/S protein or an antigenic fragment thereof;
b) a nucleotide sequence encoding an HBV HBc protein or an antigenic fragment thereof; and
c) a nucleotide sequence encoding a fusion of HBV HBs and HBc proteins or antigenic fragments thereof and, wherein the first infectious arenavirus is capable of eliciting a T cell response against the HBV pre-S2/S protein, the HBc protein, the fusion of HBs and HBc proteins, or an antigenic fragment thereof;

and a second infectious arenavirus expressing an HBV antigen as described herein, e.g., in which the ORF encoding the GP of the S genomic segment is substituted with a nucleotide sequence encoding the HBV antigen, wherein the nucleotide sequence is selected from the group consisting of:

a) a nucleotide sequence encoding an HBV pre-S2/S protein or an antigenic fragment thereof;
b) a nucleotide sequence encoding an HBV HBc protein or an antigenic fragment thereof; and
c) a nucleotide sequence encoding a fusion of HBV HBs and HBc proteins or antigenic fragments thereof; and wherein the second infectious arenavirus is capable of eliciting a T cell response against the HBV pre-S2/S protein, the HBc protein the fusion of HBs and HBc proteins, or an antigenic fragment thereof.

[0250] In certain embodiments, the first and second infectious arenaviruses are replication-deficient. In certain embodiments, the first and second infectious arenaviruses are replication-competent. In certain embodiments, either the first or second infectious arenavirus is replication-deficient. In certain embodiments, the first and second infectious arenaviruses are bisegmented. In certain embodiments, the first and second infectious

arenaviruses are trisegmented. In certain embodiments, either the first or second infectious arenavirus is bisegmented, and the other is trisegmented.

[0251] In specific embodiments, methods for treating and/or preventing an HBV infection comprise administering a first infectious arenavirus expressing a first HBV antigen, selected from: an HBV pre-S2/S protein or an antigenic fragment thereof; an HBV HBc protein or an antigenic fragment thereof, and a second infectious arenavirus expressing a second HBV antigen, selected from: an HBV pre-S2/S protein or an antigenic fragment thereof; an HBV HBc protein or an antigenic fragment thereof.

[0252] In certain embodiments, methods for treating and/or preventing an infection comprise administering two arenavirus vector constructs expressing an HBV antigen as described herein. In a specific embodiment, the two arenavirus vector constructs express a different HBV antigen.

[0253] In certain embodiments, methods for treating and/or preventing an infection comprise administering two or more arenavirus vector constructs expressing an HBV antigen as described herein. In a specific embodiment, methods for treating and/or preventing an infection comprise administering three or more arenavirus vector constructs expressing an HBV antigen as described herein. In certain embodiments, the arenavirus vector construct can be based on LCMV.

[0254] In certain embodiments, methods for treating and/or preventing an infection comprise administering two or more arenavirus vector constructs each expressing a different HBV antigen as described herein. In a specific embodiment, methods for treating and/or preventing an infection comprise administering three or more arenavirus vector constructs, each expressing a different HBV antigen as described herein. In certain embodiments, the arenavirus vector construct can be based on LCMV.

[0255] In specific embodiments, the antigen is the HBV pre-S2/S protein or a fragment thereof. (See, e.g., Section 6.2(a)).

[0256] In certain embodiments, the antigen is the HBV HBc protein or a fragment thereof. (See, e.g., Section 6.2(b)).

[0257] In certain embodiments, the antigen is the HBV HBs protein or a fragment thereof. (See, e.g., Section 6.2(c)).

[0258] In certain embodiments, the antigen is a fusion of HBV HBs and HBc proteins or antigenic fragments thereof. (See, e.g., Section 6.2(d)).

[0259] In certain embodiments, the antigen is the HBV HBe protein or a fragment thereof. (See, e.g., Section 6.2(e)).

[0260] In certain embodiments, the vector generated to encode one or more HBV antigens as described herein comprises one or more nucleic acids encoding an HBV antigen and combinations thereof as described. In specific embodiments the HBV antigens as described herein are separated by various linkers, spacers, and cleavage sites as described herein.

[0261] In another embodiment, the vector generated to encode one or more HBV antigens as described herein of the first infectious arenavirus may be based on LCMV Clone 13 or LCMV MP strain. (See, e.g., Section 7.1).

[0262] In another embodiment, the vector generated to encode one or more HBV antigens as described herein of the second infectious arenavirus may be based on LCMV Clone 13 or LCMV MP strain. (See, e.g., Section 7.1).In another embodiment, the vector generated to encode one or more HBV antigens as described herein of the first infectious arenavirus may be based on Junin virus.

[0263] In another embodiment, the vector generated to encode one or more HBV antigens as described herein of the second infectious arenavirus may be based on Junin virus.

[0264] In a specific embodiment, methods of treating and/or preventing an infection in a subject comprise administering to the subject a first infectious arenavirus expressing an HBV pre-S2/S protein or an antigenic fragment thereof and a second infectious arenavirus expressing an HBV HBc protein or an antigenic fragment thereof.

[0265] In a specific embodiment, methods of treating and/or preventing an infection in a subject comprise administering sequentially to the subject a first infectious arenavirus expressing an HBV pre-S2/S protein or an antigenic fragment thereof and a second infectious arenavirus expressing an HBV HBs protein or an antigenic fragment thereof.

[0266] In a specific embodiment, methods of treating and/or preventing an infection in a subject comprise administering simultaneously to the subject a first infectious arenavirus expressing an HBV HBc protein or an antigenic fragment thereof and a second infectious arenavirus expressing an HBV HBs protein or an antigenic fragment thereof.

[0267] In a specific embodiment, methods of treating and/or preventing an infection in a subject comprise administering sequentially to the subject a first infectious arenavirus expressing an HBV pre-S2/S protein or an antigenic fragment thereof and a second infectious arenavirus expressing a fusion of HBV HBs and HBc proteins or antigenic fragments thereof.

[0268] In a specific embodiment, methods of treating and/or preventing an infection in a subject comprise administering sequentially to the subject a first infectious arenavirus

expressing an HBV HBc protein or an antigenic fragment thereof and a second infectious arenavirus expressing a fusion of HBV HBs and HBc proteins or antigenic fragments thereof.

[0269] In a specific embodiment, methods of treating and/or preventing an infection in a subject comprise administering sequentially to the subject a first infectious arenavirus expressing an HBV HBc protein or an antigenic fragment thereof and a second infectious arenavirus expressing an HBV pre-S2/S protein or an antigenic fragment thereof.

[0270] In a specific embodiment, methods of treating and/or preventing an infection in a subject comprise administering sequentially to the subject a first infectious arenavirus expressing a fusion of HBV HBs and HBc proteins or antigenic fragments thereof and a second infectious arenavirus expressing an HBV pre-S2/S protein or an antigenic fragment thereof.

[0271] In a specific embodiment, methods of treating and/or preventing an infection in a subject comprise administering sequentially to the subject a first infectious arenavirus expressing a fusion of HBV HBs and HBc proteins or antigenic fragments thereof and a second infectious arenavirus expressing an HBV HBe protein or an antigenic fragment thereof.

[0272] In a specific embodiment, methods of treating and/or preventing an infection in a subject comprise administering sequentially to the subject a first infectious arenavirus expressing a fusion of HBV HBs and HBc proteins or antigenic fragments thereof and a second infectious arenavirus expressing an HBV HBc protein or an antigenic fragment thereof.

[0273] In a specific embodiment, methods of treating and/or preventing an infection in a subject comprise administering to the subject a first infectious arenavirus expressing an HBV pre-S2/S protein or an antigenic fragment thereof and a second infectious arenavirus expressing an HBV HBe protein or an antigenic fragment thereof.

[0274] In a specific embodiment, methods of treating and/or preventing an infection in a subject comprises administering sequentially to the subject a first infectious arenavirus expressing an HBV HBe protein or an antigenic fragment thereof and a second infectious arenavirus expressing a fusion of HBV HBs and HBc proteins or antigenic fragments thereof.

[0275] In a specific embodiment, methods of treating and/or preventing an infection in a subject comprises administering sequentially to the subject a first infectious arenavirus expressing an HBV HBs protein or an antigenic fragment thereof and a second infectious

arenavirus expressing a fusion of HBV HBs and HBc proteins or antigenic fragments thereof.

[0276] In a specific embodiment, methods of treating and/or preventing an infection in a subject comprise administering sequentially to the subject a first infectious arenavirus expressing an HBV HBe protein or an antigenic fragment thereof and a second infectious arenavirus expressing an HBV pre-S2/S protein or an antigenic fragment thereof.

[0277] In a specific embodiment, methods of treating and/or preventing an infection in a subject comprise administering sequentially to the subject a first infectious arenavirus expressing a fusion of HBV HBs and HBc proteins or antigenic fragments thereof and a second infectious arenavirus expressing an HBV HBs protein or an antigenic fragment thereof.

[0278] In a specific embodiment, methods of treating and/or preventing an infection in a subject comprise administering sequentially to the subject a first infectious arenavirus expressing an HBV HBs protein or an antigenic fragment thereof and a second infectious arenavirus expressing an HBV pre-S2/S protein or an antigenic fragment thereof.

[0279] In a specific embodiment, methods of treating and/or preventing an infection in a subject comprise administering simultaneously to the subject a first infectious arenavirus expressing an HBV HBc protein or an antigenic fragment thereof and a second infectious arenavirus expressing an HBV HBe protein or an antigenic fragment thereof.

[0280] In a specific embodiment, methods of treating and/or preventing an infection in a subject comprise administering simultaneously to the subject a first infectious arenavirus expressing an HBV HBs protein or an antigenic fragment thereof and a second infectious arenavirus expressing an HBV HBc protein or an antigenic fragment thereof.

[0281] In a specific embodiment, methods of treating and/or preventing an infection in a subject comprises administering simultaneously to the subject a first infectious arenavirus expressing an HBV HBe protein or an antigenic fragment thereof and a second infectious arenavirus expressing an HBV HBc protein or an antigenic fragment thereof.

[0282] In another embodiment, the first infectious arenavirus expressing an HBV antigen is a primary vaccine antigen and the second infectious arenavirus expressing another HBV antigen is a secondary vaccine antigen.

[0283] In certain embodiments, administering a first infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or an HBV HBc protein and a second infectious arenavirus expressing an HBV pre-S2/S protein or an HBV HBc protein provides a better protective effect to HBV after vaccination than administering a single infectious arenavirus expressing an HBV antigen, e.g., expressing only the pre-S2/S

protein (or a fragment thereof) or only the HBc protein. In other embodiments, administering a first infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or an HBV HBc protein and a second infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or an HBV HBc protein elicits a greater immune response than administering a single infectious arenavirus expressing an HBV antigen e.g., expressing only the pre-S2/S protein (or a fragment thereof) or only the HBc protein. In another embodiment, administering a first infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or an HBV HBc protein and a second infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof, or an HBV HBc protein elicits a larger CD8+ T cell response than administering a single infectious arenavirus expressing an HBV antigen e.g., expressing only the pre-S2/S protein (or a fragment thereof) or only the HBc protein. In other embodiments, administering a first infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or an HBV HBc protein and a second infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or an HBV HBc protein elicits higher titers of neutralizing antibodies than administering a single infectious arenavirus expressing an HBV antigen e.g., expressing only the pre-S2/S protein (or a fragment thereof) or only the HBc protein.

[0284] In certain embodiments, administering a first infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or an HBV HBs protein and a second infectious arenavirus expressing an HBV pre-S2/S protein or an HBV HBs protein provides a better protective effect to HBV after vaccination than administering a single infectious arenavirus expressing an HBV antigen, e.g., expressing only the pre-S2/S protein (or a fragment thereof) or only the HBs protein. In other embodiments, administering a first infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or an HBV HBs protein and a second infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or an HBV HBs protein elicits a greater immune response than administering a single infectious arenavirus expressing an HBV antigen e.g., expressing only the pre-S2/S protein (or a fragment thereof) or only the HBs protein. In another embodiment, administering a first infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or an HBV HBs protein and a second infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof, or an HBV HBs protein elicits a larger CD8+ T cell response than administering a single infectious arenavirus expressing an HBV antigen e.g., expressing only the pre-S2/S protein (or a fragment thereof) or only the HBs protein. In other embodiments,

administering a first infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or an HBV HBs protein and a second infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or an HBV HBs protein elicits higher titers of neutralizing antibodies than administering a single infectious arenavirus expressing an HBV antigen e.g., expressing only the pre-S2/S protein (or a fragment thereof) or only the HBs protein.

[0285] In certain embodiments, administering a first infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or a fusion of HBV HBs and HBc proteins and a second infectious arenavirus expressing an HBV pre-S2/S protein or a fusion of HBV HBs and HBc proteins provides a better protective effect to HBV after vaccination than administering a single infectious arenavirus expressing an HBV antigen, e.g., expressing only the pre-S2/S protein (or a fragment thereof) or only the fusion of HBV HBs and HBc proteins. In other embodiments, administering a first infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or a fusion of HBV HBs and HBc proteins and a second infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or a fusion of HBV HBs and HBc proteins elicits a greater immune response than administering a single infectious arenavirus expressing an HBV antigen e.g., expressing only the pre-S2/S protein (or a fragment thereof) or only the fusion of HBV HBs and HBc proteins. In another embodiment, administering a first infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or a fusion of HBV HBs and HBc proteins and a second infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof, or a fusion of HBV HBs and HBc proteins elicits a larger CD8+ T cell response than administering a single infectious arenavirus expressing an HBV antigen e.g., expressing only the pre-S2/S protein (or a fragment thereof) or only the fusion of HBV HBs and HBc proteins. In other embodiments, administering a first infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or a fusion of HBV HBs and HBc proteins and a second infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or a fusion of HBV HBs and HBc proteins elicits higher titers of neutralizing antibodies than administering a single infectious arenavirus expressing an HBV antigen e.g., expressing only the pre-S2/S protein (or a fragment thereof) or only the fusion of HBV HBs and HBc proteins.

[0286] In certain embodiments, administering a first infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or an HBV HBe protein and a second infectious arenavirus expressing an HBV pre-S2/S protein or an HBV HBe protein provides a better protective effect to HBV after vaccination than administering a single

infectious arenavirus expressing an HBV antigen, e.g., expressing only the pre-S2/S protein (or a fragment thereof) or only the HBe protein. In other embodiments, administering a first infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or an HBV HBe protein and a second infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or an HBV HBe protein elicits a greater immune response than administering a single infectious arenavirus expressing an HBV antigen e.g., expressing only the pre-S2/S protein (or a fragment thereof) or only the HBe protein. In another embodiment, administering a first infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or an HBV HBe protein and a second infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof, or an HBV HBe protein elicits a larger CD8+ T cell response than administering a single infectious arenavirus expressing an HBV antigen e.g., expressing only the pre-S2/S protein (or a fragment thereof) or only the HBe protein. In other embodiments, administering a first infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or an HBV HBe protein and a second infectious arenavirus expressing an HBV pre-S2/S protein or a fragment thereof or an HBV HBe protein elicits higher titers of neutralizing antibodies than administering a single infectious arenavirus expressing an HBV antigen e.g., expressing only the pre-S2/S protein (or a fragment thereof) or only the HBe protein.

[0287] In yet another embodiment, provided herein is the combined use of the replication-deficient arenavirus expressing an HBV antigen described herein and one or more replication-defective virus vectors. In a more specific embodiment the replication-defective virus vector is selected from the group comprising of poxviruses, adenoviruses, alphaviruses, herpes simplex viruses, paramyxoviruses, rhabdoviruses, poliovirus, adeno-associated virus, and sendai virus, and mixtures thereof. In a specific embodiment, the poxvirus is a modified vaccine Ankara.

[0288] In yet another embodiment, provided herein is the combined use of the replication-deficient arenavirus expressing an HBV antigen described herein and one or more replication-defective virus vectors expressing an HBV antigen. In a more specific embodiment the replication-defective virus vector is selected from the group comprising of poxviruses, adenoviruses, alphaviruses, herpes simplex viruses, paramyxoviruses, rhabdoviruses, poliovirus, adeno-associated virus, and sendai virus, and mixtures thereof. In a specific embodiment, the poxvirus is a modified vaccine Ankara.

[0289] In another embodiment, the first infectious arenavirus expressing an HBV antigen as described herein is administered before or after the second infectious arenavirus

expressing an HBV antigen as described herein. For example the first infectious arenavirus expressing an HBV antigen is administered around 30-60 minutes before or after the first administration of the second infectious arenavirus.

[0290] In another embodiment, the first infectious arenavirus expressing a vaccine antigen is administered before the second infectious arenavirus expressing a vaccine antigen. In certain embodiments there is a period of about 1 hour, 2 hours, 3 hours, 6 hours, 12 hours, 1 day, 2 days, 3 days, 5 days, 1 week, 2 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year between the administration of the first infectious arenavirus and the second infectious arenavirus.

[0291] In another embodiment, two infectious arenaviruses are administered in a treatment regime at molar ratios ranging from about 1:1 to 1:1000, in particular including: 1:1 ratio, 1:2 ratio, 1:5 ratio, 1:10 ratio, 1:20 ratio, 1:50 ratio, 1:100 ratio, 1:200 ratio, 1:300 ratio, 1:400 ratio, 1:500 ratio, 1:600 ratio, 1:700 ratio, 1:800 ratio, 1:900 ratio, 1:1000 ratio.

[0292] In another embodiment, the subjects whom two or more infectious arenaviruses expressing an HBV antigen described herein are administered have, are susceptible to, or are at risk for an HBV infection. In another embodiment, the subjects whom two or more infectious arenaviruses expressing an HBV antigen described herein are administered are infected with, are susceptible to, or are at risk for, an infection with HBV.

[0293] In another embodiment, the subjects whom two or more infectious arenaviruses expressing an HBV antigen described herein, are administered simultaneously have, are susceptible to, or are at risk for an HBV infection. In another embodiment, the subjects whom two or more infectious arenaviruses expressing an HBV antigen described herein are administered simultaneously are infected with, are susceptible to, or are at risk for, an infection with HBV.

[0294] In another embodiment, the subjects whom two or more infectious arenaviruses expressing an HBV antigen described herein, are administered sequentially have, are susceptible to, or are at risk for an HBV infection. In another embodiment, the subjects whom two or more infectious arenaviruses expressing an HBV antigen described herein are administered sequentially are infected with, are susceptible to, or are at risk for, an infection with HBV.

[0295] In another embodiment, said two or more infectious arenaviruses expressing an HBV antigen as described herein are further administered in combination with at least one other medicament for treating and/or preventing HBV. Therapeutic medicaments for treating and/or preventing HBV include, but are not limited to entecavir (BARACLUDE®;

Bristol-Myers Squibb), lamivudine (EPIVIR HBV®; GlaxoSmithKline), adefovir dipivoxil (HEPSERA®; Gilead Sciences), interferon alpha 2b (INTRON A®; Schering), pegylated interferon (PEGASYS®; Roche), telbivudine (TYZEKA®, Novartis), and tenofovir (VIREAD®; Gilead Sciences).

[0296] In another embodiment, said two or more infectious arenaviruses expressing an HBV antigen as described herein are further administered in a combination with at least one other immunomodulator. In a more specific embodiment, said two or more infectious arenaviruses expressing an HBV antigen as described herein are further administered in a combination with at least one Th1-specific adjuvant. In a more specific embodiment the Th-1 specific adjuvant is Bacillus Calmette-Guerin (BCG).

[0297] In another embodiment, the administration regime can involve administering to a symptomatic subject a second infectious arenavirus expressing an HBV antigen as described herein. In yet another embodiment, the administration regime can involve administering to an subject with a compromised immune system, especially transplant recipients, HIV-infected persons, a pregnant subject, a subject who has cancer, a second infectious arenavirus expressing an HBV antigen as described herein. In another embodiment, two or more infectious arenaviruses expressing an HBV antigen as described herein are administered to a subject who is a child of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 years of age suffering from or susceptible to, or at risk for, an infection with HBV.

[0298] In another embodiment, the administration regime can involve administering to a subject who is a child, a first arenavirus expressing an HBV antigen, and administering to the same subject who is an adolescent a second arenavirus expressing an HBV antigen. In a specific embodiment, the administration regime can involve administering to a subject who is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 years of age a first arenavirus expressing an HBV antigen as described herein, and to the same subject who is 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 years of age a second infectious arenavirus expressing an HBV antigen.

[0299] In another embodiment, the administration regime can involve administering to a prepubescent subject a second infectious arenavirus expressing an HBV antigen. In another embodiment, the administration regime can involve administering to an adolescent male, aged 12 to 18 years a second infectious arenavirus expressing an HBV antigen as described herein. In another embodiment, the administration regime can involve administering to a female, aged 12 to 18 years a second infectious arenavirus expressing an HBV antigen.

[0300] In another embodiment, administering two or more infectious arenaviruses expressing an HBV antigen reduces the risk that an individual will develop an infection with HBV by at least 10%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the risk of developing an infection with HBV in the absence of such treatment.

[0301] In another embodiment, administering two or more infectious arenaviruses expressing an HBV antigen, administered separately, reduces the risk that an individual will develop an infection with HBV by at least 10%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the risk of developing an infection with HBV in the absence of such treatment.

[0302] In another embodiment, administering two or more infectious arenaviruses expressing an HBV antigen, administered sequentially, reduces the risk that an individual will develop an infection with HBV by at least 10%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the risk of developing an infection with HBV in the absence of such treatment.

[0303] Without being limited by theory, administration of a first infectious arenavirus and subsequently of a second infectious arenavirus vector results in a prime-boost effect.

[0304] In certain embodiments, provided herein are methods for treating and/or preventing an HBV infection comprising administering two or more arenavirus vector constructs each expressing the same or a different HBV antigen sequentially. The time interval between each administration can be about 1 week, about 2 weeks, about 3 week, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 18 months, or about 24 months.

[0305] In certain embodiments, the first infectious arenavirus and the second infectious arenavirus are homologous. In certain embodiments, the first infectious arenavirus and the second infectious arenavirus are heterologous.

[0306] In certain specific embodiments, the first infectious arenavirus is an Old World arenavirus, and the second infectious arenavirus is an Old World arenavirus. In certain specific embodiments, the first infectious arenavirus is an Old World arenavirus, and the second infectious arenavirus is a New World arenavirus. In certain specific embodiments,

the first infectious arenavirus is a New World arenavirus, and the second infectious arenavirus is a New World arenavirus. In certain specific embodiments, the first infectious arenavirus is a New World arenavirus, and the second infectious arenavirus is an Old World arenavirus.

[0307] In certain specific embodiments, the first infectious arenavirus is derived from LCMV, and the second infectious arenavirus is derived from LCMV. In certain specific embodiments, the first infectious arenavirus is derived from LCMV, and the second infectious arenavirus is derived from Junin virus. In certain specific embodiments, the first infectious arenavirus is derived from Junin virus, and the second infectious arenavirus is derived from Junin virus. In certain specific embodiments, the first infectious arenavirus is derived from Junin virus, and the second infectious arenavirus is derived from LCMV.

[0308] In certain embodiments, provided herein is a method of treating and/or preventing an HBV infection wherein a first infectious arenavirus is administered first as a "prime," and a second infectious arenavirus is administered as a "boost." The first and the second infectious arenavirus vectors can express the same or different HBV antigens. In certain specific embodiments, the "prime" administration is performed with an infectious arenavirus derived from LCMV, and the "boost" is performed with an infectious arenavirus derived from Junin virus. In certain specific embodiments, the "prime" administration is performed with an infectious arenavirus derived from Junin virus, and the "boost" is performed with an infectious arenavirus derived from LCMV.

[0309] In certain embodiments, administering a first infectious arenavirus expressing an HBV antigen or a fragment thereof, followed by administering a second infectious arenavirus expressing an HBV antigen or a fragment thereof results in a greater antigen specific CD8+ T cell response than administering a single infectious arenavirus expressing an HBV antigen or a fragment thereof. In certain embodiments, the antigen specific CD8+ T cell count increases by 50%, 100%, 150% or 200% after the second administration compared to the first administration. In certain embodiments, administering a third infectious arenavirus expressing an HBV antigen results in a greater antigen specific CD8+ T cell response than administering two consecutive infectious arenaviruses expressing an HBV antigen. In certain embodiments, the antigen specific CD8+ T cell count increases by about 50%, about 100%, about 150%, about 200% or about 250% after the third administration compared to the first administration.

[0310] In certain embodiments, provided herein are methods for treating and/or preventing an infection comprising administering two or more arenavirus vector constructs, wherein the two or more arenavirus vector constructs are homologous, and

wherein the time interval between each administration is about 1 week, about 2 weeks, about 3 week, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 18 months, or about 24 months.

[0311] In certain embodiments, administering a first infectious arenavirus expressing an HBV antigen or a fragment thereof and a second, heterologous, infectious arenavirus expressing an HBV antigen or a fragment thereof elicits a greater CD8+ T cell response than administering a first infectious arenavirus expressing an HBV antigen or a fragment thereof and a second, homologous, infectious arenavirus expressing an HBV antigen or a fragment thereof.

[0312] In certain specific embodiments, the first infectious arenavirus expressing an HBV pre-S2/S protein is LCMV, and the second, heterologous, infectious arenavirus expressing an HBV pre-S2/S protein is Junin virus. In certain specific embodiments, the first infectious arenavirus expressing an HBV pre-S2/S protein is Junin virus, and the second, heterologous, infectious arenavirus expressing an HBV pre-S2/S protein is LCMV.

[0313] In certain specific embodiments, the first infectious arenavirus expressing an HBV HBc protein is LCMV, and the second, heterologous, infectious arenavirus expressing an HBV HBc protein is Junin virus. In certain specific embodiments, the first infectious arenavirus expressing an HBV HBc protein is Junin virus, and the second, heterologous, infectious arenavirus expressing an HBV HBc protein is LCMV.

[0314] In certain specific embodiments, the first infectious arenavirus expressing an HBV HBs and HBc fusion protein is LCMV, and the second, heterologous, infectious arenavirus expressing an HBV HBs and HBc fusion protein is Junin virus. In certain specific embodiments, the first infectious arenavirus expressing an HBV HBs and HBc fusion protein is Junin virus, and the second, heterologous, infectious arenavirus expressing an HBV HBs and HBc fusion protein is LCMV.

[0315] In certain specific embodiments, administering a first infectious arenavirus expressing an HBV pre-S2/S protein and a second, heterologous, infectious arenavirus expressing an HBV pre-S2/S protein elicits a greater CD8+ T cell response than administering a first infectious arenavirus expressing an HBV pre-S2/S protein and a second, homologous, infectious arenavirus expressing HBV pre-S2/S protein. In certain specific embodiments, administering a first infectious arenavirus expressing an HBV pre-S2/S protein and a second, heterologous, infectious arenavirus expressing an HBV pre-

S2/S protein elicits a CD8+ T cell response that is about 20%, about 40%, about 60%, about 80%, about 100%, about 120%, about 140%, about 160%, about 180%, or about 200% greater than administering a first infectious arenavirus expressing an HBV pre-S2/S protein and a second, homologous, infectious arenavirus expressing an HBV pre-S2/S protein.

[0316] In certain specific embodiments, administering a first infectious arenavirus expressing an HBV HBc protein and a second, heterologous, infectious arenavirus expressing an HBV HBc protein elicits a greater CD8+ T cell response than administering a first infectious arenavirus expressing an HBV HBc protein and a second, homologous, infectious arenavirus expressing HBV HBc protein. In certain specific embodiments, administering a first infectious arenavirus expressing an HBV HBc protein and a second, heterologous, infectious arenavirus expressing an HBV HBc protein elicits a CD8+ T cell response that is about 20%, about 40%, about 60%, about 80%, about 100%, about 120%, about 140%, about 160%, about 180%, or about 200% greater than administering a first infectious arenavirus expressing an HBV HBc protein and a second, homologous, infectious arenavirus expressing an HBV HBc protein.

[0317] In certain specific embodiments, administering a first infectious arenavirus expressing an HBV HBs and HBc fusion protein and a second, heterologous, infectious arenavirus expressing an HBV HBs and HBc fusion protein elicits a greater CD8+ T cell response than administering a first infectious arenavirus expressing an HBV HBs and HBc fusion protein and a second, homologous, infectious arenavirus expressing HBV HBs and HBc fusion protein. In certain specific embodiments, administering a first infectious arenavirus expressing an HBV HBs and HBc fusion protein and a second, heterologous, infectious arenavirus expressing an HBV HBs and HBc fusion protein elicits a CD8+ T cell response that is about 20%, about 40%, about 60%, about 80%, about 100%, about 120%, about 140%, about 160%, about 180%, or about 200% greater than administering a first infectious arenavirus expressing an HBV HBs and HBc fusion protein and a second, homologous, infectious arenavirus expressing an HBV HBs and HBc fusion protein.

[0318] In certain embodiments, provided herein are methods for treating and/or preventing an infection comprising administering two or more arenavirus vector constructs, wherein the two or more arenavirus vector constructs are heterologous, and wherein the time interval between each administration is about 1 week, about 2 weeks, about 3 week, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 3 months, about 4 months, about 5 months, about 6 months, about 7

months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 18 months, or about 24 months.

[0319] In yet another embodiment, provided herein is the combined use of the replication-deficient arenavirus expressing an HBV antigen described herein and one or more replication-defective virus vectors. In a more specific embodiment the replication-defective virus vector is selected from the group comprising of poxviruses, adenoviruses, alphaviruses, herpes simplex viruses, paramyxoviruses, rhabdoviruses, poliovirus, adeno-associated virus, and sendai virus, and mixtures thereof. In a specific embodiment, the poxvirus is a modified vaccine Ankara.

[0320] In yet another embodiment, provided herein is the combined use of the replication-deficient arenavirus expressing an HBV antigen described herein and one or more replication-defective virus vectors expressing an HBV antigen. In a more specific embodiment the replication-defective virus vector is selected from the group comprising of poxviruses, adenoviruses, alphaviruses, herpes simplex viruses, paramyxoviruses, rhabdoviruses, poliovirus, adeno-associated virus, and sendai virus, and mixtures thereof. In a specific embodiment, the poxvirus is a modified vaccine Ankara.

[0321] In another embodiment, the first infectious arenavirus expressing an HBV antigen as described herein is administered before or after the second infectious arenavirus expressing an HBV antigen as described herein. For example the first infectious arenavirus expressing an HBV antigen is administered around 30-60 minutes before or after the first administration of the second infectious arenavirus.

[0322] In another embodiment, the first infectious arenavirus expressing a vaccine antigen is administered before the second infectious arenavirus expressing a vaccine antigen. In certain embodiments there is a period of about 1 hour, 2 hours, 3 hours, 6 hours, 12 hours, 1 day, 2 days, 3 days, 5 days, 1 week, 2 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year between the administration of the first infectious arenavirus and the second infectious arenavirus.

[0323] In another embodiment, two infectious arenaviruses are administered in a treatment regime at molar ratios ranging from about 1:1 to 1:1000, in particular including: 1:1 ratio, 1:2 ratio, 1:5 ratio, 1:10 ratio, 1:20 ratio, 1:50 ratio, 1:100 ratio, 1:200 ratio, 1:300 ratio, 1:400 ratio, 1:500 ratio, 1:600 ratio, 1:700 ratio, 1:800 ratio, 1:900 ratio, 1:1000 ratio.

[0324] In another embodiment, the subjects to whom two or more infectious arenaviruses expressing an HBV antigen described herein are administered have, are susceptible to, or are at risk for an HBV infection. In another embodiment, the subjects to whom two or

more infectious arenaviruses expressing an HBV antigen described herein are administered are infected with, are susceptible to, or are at risk for, an infection with HBV.

[0325] The subjects who can be treated with the methods provided herein are susceptible to, or are at risk for an HBV infection.

[0326] In another embodiment, said two or more infectious arenaviruses expressing an HBV antigen as described herein further express at least another immunostimulatory peptide, polypeptide or protein. In certain embodiments, the immunostimulatory peptide, polypeptide or protein is Calreticulin (CRT), or a fragment thereof; Ubiquitin or a fragment thereof; Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), or a fragment thereof; Invariant chain (CD74) or an antigenic fragment thereof; Mycobacterium tuberculosis Heat shock protein 70 or an antigenic fragment thereof; Herpes simplex virus 1 protein VP22 or an antigenic fragment thereof; CD40 ligand or an antigenic fragment thereof; or Fms-related tyrosine kinase 3 (Flt3) ligand or an antigenic fragment thereof.

[0327] Heterologous prime-boost methods with infectious replication-defective arenavirus vectors wherein the two infectious replication-defective arenavirus vectors are derived from different arenaviruses (*e.g.*, LCMV and Junin virus) are also provided. These infectious replication-defective arenavirus vectors can express an antigen, such as an antigen of HBV.

[0328] Heterologous prime-boost methods with infectious replication-competent arenavirus vectors wherein the two infectious replication-competent arenavirus vectors are derived from different arenaviruses (*e.g.*, LCMV and Junin virus) are also provided. These infectious replication-competent arenavirus vectors can express an antigen, such as an antigen of HBV.

6.8 (b) Compositions

[0329] The invention furthermore relates to vaccines, immunogenic compositions, and pharmaceutical compositions comprising a genetically engineered arenavirus as described herein. Such vaccines and pharmaceutical compositions can be formulated according to standard procedures in the art.

[0330] In one embodiment, provided herein are compositions comprising two or more infectious arenaviruses expressing an HBV antigen as described herein. See, e.g., Section 6.2.

[0331] In a specific embodiments, the compositions described herein are for use in methods discussed previously, wherein the methods comprise administering to a subject

a first infectious arenavirus expressing an HBV antigen as described herein, e.g., in which the ORF encoding the GP of the S genomic segment is substituted with a nucleotide sequence encoding the HBV antigen. The HBV antigen can be but is not limited to:

a) an HBV pre-S2/S protein or an antigenic fragment thereof;

b) an HBV HBc protein or an antigenic fragment thereof; and

c) a fusion of HBV HBs and HBc proteins or antigenic fragments thereof

The methods further comprise administering a second infectious arenavirus composition expressing an HBV antigen as described herein, e.g., in which the ORF encoding the GP of the S genomic segment is substituted with a nucleotide sequence encoding the HBV antigen. The HBV antigen can be but is not limited to:

a) an HBV pre-S2/S protein or an antigenic fragment thereof;

b) an HBV HBc protein or an antigenic fragment thereof; and

c) a fusion of HBV HBs and HBc proteins or antigenic fragments thereof

In certain embodiments, the first and second infectious arenaviruses are replication-deficient. In certain embodiments, the first and second infectious arenaviruses are replication-competent. In certain embodiments, either the first or second infectious arenavirus is replication-deficient.

[0332] In specific embodiments, methods for treating and/or preventing an HBV infection comprise administering a first infectious arenavirus expressing a first HBV antigen, selected from: an HBV pre-S2/S protein or an antigenic fragment thereof; an HBV HBc protein or an antigenic fragment thereof; a fusion of HBV HBs and HBc proteins or antigenic fragments thereof, , as described herein and a second infectious arenavirus expressing a second HBV antigen, selected from: an HBV pre-S2/S protein or an antigenic fragment thereof; an HBV HBc protein or an antigenic fragment thereof; a fusion of HBV HBs and HBc proteins or antigenic fragments thereof.

[0333] In certain embodiments, provided herein are compositions suitable for use in a method of treating and/or preventing an HBV infection comprising administering two arenavirus constructs expressing an HBV antigen as described herein. In a specific embodiment, the two arenavirus vector constructs express an HBV antigen.

[0334] In certain embodiments, provided herein are compositions comprising two or more arenavirus vector constructs expressing an HBV antigen as described herein. In specific embodiments, provided herein are compositions comprising three or more arenavirus vector constructs expressing an HBV antigen as described herein. In certain embodiments, the arenavirus can be LCMV.

[0335] In specific embodiments, the antigen is the HBV pre-S2/S protein or a fragment thereof. (See, e.g., Section 6.2(a)).

[0336] In certain embodiments, the antigen is the HBV HBc protein or a fragment thereof. (See, e.g., Section 6.2(b)).

[0337] In certain embodiments, the antigen is the HBV HBs protein or a fragment thereof. (See, e.g., Section 6.2(c)).

[0338] In certain embodiments, the antigen is a fusion of the HBV HBs and HBc proteins or antigenic fragments thereof (See, e.g., Section 6.2(d)).

[0339] In certain embodiments, the antigen is the HBV HBe protein or a fragment thereof. (See, e.g., Section 6.2(e)).

[0340] In certain embodiments, the vector generated to encode one or more HBV antigens as described herein comprises one or more nucleic acids encoding an HBV antigen and combinations thereof as described. In specific embodiments the HBV antigens as described herein are separated by various linkers, spacers, and cleavage sites as described herein.

[0341] In another embodiment, the vector generated to encode one or more HBV antigens as described herein of the first infectious arenavirus may be based on LCMV Clone 13 or LCMV MP strain. (See, e.g., Section 7.1).

[0342] In another embodiment, the vector generated to encode one or more HBV antigens as described herein of the second infectious arenavirus may be based on LCMV Clone 13 or LCMV MP strain. (See, e.g., Section 7.1).

[0343] In a specific embodiment, provided herein are compositions for use in a method of treating and/or preventing an HBV infection in a subject comprising administering to the subject a first infectious arenavirus composition expressing an HBV pre-S2/S protein or an antigenic fragment thereof and a second infectious arenavirus composition expressing an HBV HBc protein or an antigenic fragment thereof.

[0344] In a specific embodiment, provided herein are compositions for use in a method of treating and/or preventing an infection in a subject comprising administering sequentially to the subject a first infectious arenavirus expressing an HBV pre-S2/S protein or an antigenic fragment thereof and a second infectious arenavirus expressing an HBV HBs protein or an antigenic fragment thereof.

[0345] In a specific embodiment, provided herein are compositions for use in a method of treating and/or preventing an infection in a subject comprising administering simultaneously to the subject a first infectious arenavirus expressing an HBV HBc protein

or an antigenic fragment thereof and a second infectious arenavirus expressing an HBV HBs protein or an antigenic fragment thereof.

[0346] In a specific embodiment, provided herein are compositions for use in a method of treating and/or preventing an infection in a subject comprising administering sequentially to the subject a first infectious arenavirus expressing an HBV pre-S2/S protein or an antigenic fragment thereof and a second infectious arenavirus expressing a fusion of HBV HBs and HBc proteins or antigenic fragments thereof.

[0347] In a specific embodiment, provided herein are compositions for use in a method of treating and/or preventing an infection in a subject comprising administering simultaneously to the subject a first infectious arenavirus expressing an HBV HBc protein or an antigenic fragment thereof and a second infectious arenavirus expressing a fusion of HBV HBs and HBc proteins or antigenic fragments thereof.

[0348] In a specific embodiment, provided herein are compositions for use in a method of treating and/or preventing an infection in a subject comprising administering sequentially to the subject a first infectious arenavirus expressing an HBV HBc protein or an antigenic fragment thereof and a second infectious arenavirus expressing an HBV pre-S2/S protein or an antigenic fragment thereof.

[0349] In a specific embodiment, provided herein are compositions for use in a method of treating and/or preventing an infection in a subject comprising administering sequentially to the subject a first infectious arenavirus expressing a fusion of HBV HBs and HBc proteins or antigenic fragments thereof and a second infectious arenavirus expressing an HBV pre-S2/S protein or an antigenic fragment thereof.

[0350] In a specific embodiment, provided herein are compositions for use in a method of treating and/or preventing an infection in a subject comprising administering sequentially to the subject a first infectious arenavirus expressing a fusion of HBV HBs and HBc proteins or antigenic fragments thereof and a second infectious arenavirus expressing an HBV HBe protein or an antigenic fragment thereof.

[0351] In a specific embodiment, provided herein are compositions for use in a method of treating and/or preventing an infection in a subject comprising administering sequentially to the subject a first infectious arenavirus expressing a fusion of HBV HBs and HBc proteins or antigenic fragments thereof and a second infectious arenavirus expressing an HBV HBc protein or an antigenic fragment thereof.

[0352] In a specific embodiment, provided herein are compositions for use in a method of treating and/or preventing an infection in a subject comprising administering to the subject a first infectious arenavirus expressing an HBV pre-S2/S protein or an antigenic

fragment thereof and a second infectious arenavirus expressing an HBV HBe protein or an antigenic fragment thereof.

[0353] In a specific embodiment, provided herein are compositions or use in a method of treating and/or preventing an infection in a subject comprising administering sequentially to the subject a first infectious arenavirus expressing an HBV HBe protein or an antigenic fragment thereof and a second infectious arenavirus expressing a fusion of HBV HBs and HBc proteins or antigenic fragments thereof.

[0354] In a specific embodiment, provided herein are compositions for use in a method of treating and/or preventing an infection in a subject comprising administering sequentially to the subject a first infectious arenavirus expressing an HBV HBs protein or an antigenic fragment thereof and a second infectious arenavirus expressing a fusion of HBV HBs and HBc proteins or antigenic fragments thereof.

[0355] In a specific embodiment, provided herein are compositions for use in a method of treating and/or preventing an infection in a subject comprising administering sequentially to the subject a first infectious arenavirus expressing an HBV HBe protein or an antigenic fragment thereof and a second infectious arenavirus expressing an HBV pre-S2/S protein or an antigenic fragment thereof.

[0356] In a specific embodiment, provided herein are compositions for use in a method of treating and/or preventing an infection in a subject comprising administering sequentially to the subject a first infectious arenavirus expressing a fusion of HBV HBs and HBc proteins or antigenic fragments thereof and a second infectious arenavirus expressing an HBV HBs protein or an antigenic fragment thereof.

[0357] In a specific embodiment, provided herein are compositions for use in a method of treating and/or preventing an infection in a subject comprising administering sequentially to the subject a first infectious arenavirus expressing an HBV HBs protein or an antigenic fragment thereof and a second infectious arenavirus expressing an HBV pre-S2/S protein or an antigenic fragment thereof.

[0358] In a specific embodiment, provided herein are compositions for use in a method of treating and/or preventing an infection in a subject comprising administering simultaneously to the subject a first infectious arenavirus expressing an HBV HBc protein or an antigenic fragment thereof and a second infectious arenavirus expressing an HBV HBe protein or an antigenic fragment thereof.

[0359] In a specific embodiment, provided herein are compositions for use in a method of treating and/or preventing an infection in a subject comprising administering simultaneously to the subject a first infectious arenavirus expressing an HBV HBs protein

or an antigenic fragment thereof and a second infectious arenavirus expressing an HBV HBc protein or an antigenic fragment thereof.

[0360] In a specific embodiment, provided herein are compositions four use in a method of treating and/or preventing an infection in a subject comprising administering simultaneously to the subject a first infectious arenavirus expressing an HBV HBe protein or an antigenic fragment thereof and a second infectious arenavirus expressing an HBV HBc protein or an antigenic fragment thereof.

[0361] In another embodiment, the first infectious arenavirus composition expressing an HBV antigen is a primary vaccine antigen and the second infectious arenavirus expressing another HBV antigen is a secondary vaccine antigen.

[0362] In yet another embodiment, provided herein is the combined use of the replication-deficient arenaviruses compositions expressing an HBV antigen as described herein and one or more replication-defective virus vector compositions. In a more specific embodiment the replication-defective virus vector composition can be but is not limited to: poxviruses, adenoviruses, alphaviruses, herpes simplex viruses, paramyxoviruses, rhabdoviruses, poliovirus, adeno-associated virus, and Sendai virus, and mixtures thereof. In a specific embodiment, the poxvirus is a modified vaccine Ankara.

[0363] In another embodiment, two infectious arenaviruses compositions have molar ratios ranging from about 1:1 to 1:1000, in particular including: 1:1 ratio, 1:2 ratio, 1:5 ratio, 1:10 ratio, 1:20 ratio, 1:50 ratio, 1:100 ratio, 1:200 ratio, 1:300 ratio, 1:400 ratio, 1:500 ratio, 1:600 ratio, 1:700 ratio, 1:800 ratio, 1:900 ratio, 1:1000 ratio.

[0364] In another embodiment, two or more infectious arenavirus compositions expressing an HBV antigen described herein are suitable for administration to subjects who have, are susceptible to, or are at risk for an HBV infection. In another embodiment, the subjects, to whom two or more infectious arenaviruses compositions expressing an HBV antigen described herein or a composition thereof is administered, are infected with, are susceptible to, or are at risk for, an infection with HBV.

[0365] In another embodiment, said two or more infectious arenavirus compositions further comprise at least one other medicament for treating and/or preventing HBV infection. Therapeutic medicaments include, but are not limited to, entecavir (BARACLUDE®; Bristol-Myers Squibb), lamivudine (EPIVIR HBV®; GlaxoSmithKline), adefovir dipivoxil (HEPSERA®; Gilead Sciences), interferon alpha 2b (INTRON A®; Schering), pegylated interferon (PEGASYS®; Roche), telbivudine (TYZEKA®, Novartis), and tenofovir (VIREAD®; Gilead Sciences).

[0366] In another embodiment, compositions are suitable for administrating to a symptomatic subject a second infectious arenavirus composition expressing an HBV antigen or a fragment thereof as described herein. In yet another embodiment, the compositions are suitable for administration to a subject with a compromised immune system, especially transplant recipients, HIV-infected persons, a pregnant subject, or a subject who has cancer, a second infectious arenavirus composition expressing an HBV antigen described herein or a fragment thereof. In another embodiment, two or more infectious arenavirus compositions expressing an HBV antigen as described herein or a fragment thereof are suitable for administrating to a subject who is a child of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 years of age suffering from or susceptible to, or are at risk for, an infection with HBV.

[0367] In another embodiment, compositions are suitable for administrating to a subject who is a child, a first arenavirus expressing an HBV antigen, and administering to the same subject who is an adolescent a second arenavirus expressing an HBV antigen. In a specific embodiment, the administration regime can involve administering to a subject who is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 years of age a first arenavirus expressing an HBV antigen as described herein, and to the same subject who is 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 years of age a second infectious arenavirus expressing an HBV antigen.

[0368] In another embodiment, compositions are suitable for administering to a prepubescent subject a second infectious arenavirus expressing an HBV antigen. In another embodiment, the administration regime can involve administering to an adolescent male, aged 12 to 18 years a second infectious arenavirus expressing an HBV antigen as described herein. In another embodiment, the administration regime can involve administering to a female, aged 12 to 18 years a second infectious arenavirus expressing an HBV antigen.

[0369] In another embodiment, two or more infectious arenavirus compositions expressing an HBV antigen or a fragment thereof, as described herein reduce the risk that an individual will develop an infection with HBV by at least 10%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the risk of developing an infection with HBV in the absence of such treatment.

[0370] In another embodiment, two or more infectious arenavirus compositions expressing an HBV antigen or a fragment thereof, as described herein, administered

separately, reduce the risk that an individual will develop an infection with HBV by at least 10%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the risk of developing an infection with HBV in the absence of such treatment.

[0371] In another embodiment, two or more infectious arenavirus compositions expressing an HBV antigen or a fragment thereof, as described herein, administered sequentially, reduce the risk that an individual will develop an infection with HBV by at least 10%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the risk of developing an infection with HBV in the absence of such treatment.

[0372] In another embodiment, provided herein the invention provides a vaccine composition comprising a synergistic combination of two or more infectious replication-deficient arenaviruses expressing an HBV antigen.

[0373] In another embodiment, provided herein the invention provides a vaccine composition comprising a synergistic combination of two or more infectious replication-competent arenaviruses expressing an HBV antigen.

6.9 Assays

[0374] Assay for Measuring Arenavirus Vector Infectivity Any assay known to the skilled artisan can be used for measuring the infectivity of an arenavirus vector preparation. For example, determination of the virus/vector titer can be done by a "focus forming unit assay" (FFU assay). In brief, complementing cells, e.g. HEK 293 cells expressing LCMV GP protein, are plated and inoculated with different dilutions of a virus/vector sample. After an incubation period, to allow cells to form a monolayer and virus to attach to cells, the monolayer is covered with Methylcellulose. When the plates are further incubated, the original infected cells release viral progeny. Due to the Methylcellulose overlay the spread of the new viruses is restricted to neighboring cells. Consequently, each infectious particle produces a circular zone of infected cells called a Focus. Such Foci can be made visible and by that countable using antibodies against LCMV- NP and a HRP-based color reaction. The titer of a virus / vector can be calculated in focus-forming units per milliliter (FFU/mL).

[0375] To determine the infectious titer (FFU/mL) of transgene-carrying vectors this assay is modified by the use of the respective transgene-specific antibody instead of anti-LCMV-NP antibody.

[0376] Serum ELISA Determination of the humoral immune response upon vaccination of animals (e.g. mice, guinea pigs) can be done by antigen-specific serum ELISAs (enzyme-linked immunosorbent assays). In brief, plates are coated with antigen (e.g. recombinant protein), blocked to avoid unspecific binding of antibodies and incubated with serial dilutions of sera. After incubation, bound serum-antibodies can be detected, e.g., using an enzyme-coupled anti-species (e.g. mouse, guinea pig)-specific antibody (detecting total IgG or IgG subclasses) and subsequent color reaction. Antibody titers can be determined as, e.g., endpoint geometric mean titer.

[0377] Immunocapture ELISA (IC-ELISA) may also be performed (*see* Shanmugham et al., 2010, Clin. Vaccine Immunol. 17(8):1252-1260), wherein the capture agents are cross-linked to beads.

[0378] Neutralizing Assay in ARPE-19 cells Determination of the neutralizing activity of induced antibodies in sera is performed with the following cell assay using ARPE-19 cells from ATCC and a GFP-tagged virus. In addition supplemental serum as a source of exogenous complement is used. The assay is started with seeding of 6.5×10^3 cells/well (50gl/well) in a 384 well plate one or two days before using for neutralization. The neutralization is done in 96-well sterile tissue culture plates without cells for 1h at 37°C. After the neutralization incubation step the mixture is added to the cells and incubated for additional 4 days for GFP-detection with a plate reader. A positive neutralizing human sera is used as assay positive control on each plate to check the reliability of all results. Titers (EC50) are determined using a 4 parameter logistic curve fitting. As additional testing the wells are checked with a fluorescence microscope.

[0379] Plaque Reduction Assay In brief, plaque reduction (neutralization) assays for Hepatitis B virus are performed by use of an isolate of HBV tagged with green fluorescent protein, 5% rabbit serum was used as a source of exogenous complement, and plaques are enumerated by fluorescence microscopy. Neutralization titers are defined as the highest dilution of serum that results in a 50% reduction in plaques, compared with that in control (pre-immune) serum samples.

[0380] Neutralization Assay in guinea pig lung fibroblast (GPL) cells In brief, serial dilutions of test and control (pre-vaccination) sera were prepared in GPL complete media with supplemental rabbit serum (1%) as a source of exogenous complement. The dilution series spanned 1:40 through 1:5120. Serum dilutions were incubated with eGFP tagged

virus (100-200 pfu per well) for 30 min at 37°C, and then transferred to 12-well plates containing confluent GPL cells. Samples were processed in triplicate. After 2 hours incubation at 37°C the cells were washed with PBS, re-fed with GPL complete media and incubated at 37°C / 5% CO_2 for 5 days. Plaques were visualized by fluorescence microscopy, counted, and compared to control wells. That serum dilution resulting in a 50% reduction in plaque number compared to controls was designated as the neutralizing titer.

[0381] qPCR LCMV RNA genomes are isolated using QIAamp Viral RNA mini Kit (QIAGEN), according to the protocol provided by the manufacturer. LCMV RNA genome equivalents are detected by quantitative PCR carried out on an StepOnePlus Real Time PCR System (Applied Biosystems) with SuperScript® III Platinum® One-Step qRT-PCR Kit (Invitrogen) and primers and probes (FAM reporter and NFQ-MGB Quencher) specific for part of the LCMV NP coding region. The temperature profile of the reaction is : 30 min at 60°C, 2 min at 95°C, followed by 45 cycles of 15 s at 95°C, 30 s at 56°C. RNA is quantified by comparison of the sample results to a standard curve prepared from a log10 dilution series of a spectrophotometrically quantified, in vitro-transcribed RNA fragment, corresponding to a fragment of the LCMV NP coding sequence containing the primer and probe binding sites.

[0382] Western Blotting Infected cells grown in tissue culture flasks or in suspension are lysed at indicated timepoints post infection using RIPA buffer (Thermo Scientific) or used directly without cell-lysis. Samples are heated to 99°C for 10 minutes with reducing agent and NuPage LDS Sample buffer (NOVEX) and chilled to room temperature before loading on 4-12% SDS-gels for electrophoresis. Proteins are blotted onto membranes using Invitrogens iBlot Gel transfer Device and visualized by Ponceau staining. Finally, the preparations are probed with an primary antibodies directed against proteins of interest and alkaline phosphatase conjugated secondary antibodies followed by staining with 1-Step NBT/BCIP solution (INVITROGEN).

[0383] MHC-Peptide Multimer Staining Assay for Detection of Antigen-Specific CD8+ T-cell proliferation Any assay known to the skilled artisan can be used to test antigen-specific CD8+ T-cell responses. For example, the MHC-peptide tetramer staining assay can be used (see, e.g., Altman J.D. et al., Science. 1996; 274:94-96; and Murali-Krishna K. et al., Immunity. 1998; 8:177-187). Briefly, the assay comprises the following steps, a tetramer assay is used to detect the presence of antigen specific T-cells. In order for a T-cell to detect the peptide to which it is specific, it must both recognize the peptide and the tetramer of MHC molecules custom made for an antigen specific T-cell (typically

fluorescently labeled). The tetramer is then detected by flow cytometry via the fluorescent label.

[0384] ELISPOT Assay for Detection of Antigen-Specific CD4+ T-cell Proliferation Any assay known to the skilled artisan can be used to test antigen-specific CD4+ T-cell responses. For example, the ELISPOT assay can be used (see, e.g., Czerkinsky C.C. et al., J Immunol Methods. 1983; 65:109-121; and Hutchings P.R. Et al., J Immunol Methods. 1989; 120:1-8). Briefly, the assay comprises the following steps: An immunospot plate is coated with an anti-cytokine antibody. Cells are incubated in the immunospot plate. Cells secrete cytokines and are then washed off. Plates are then coated with a second biotyinlated-anticytokine antibody and visualized with an avidin-HRP system.

[0385] Intracellular Cytokine Assay for Detection of Functionality of CD8+ and CD4+ T-cell Responses Any assay known to the skilled artisan can be used to test the functionality of CD8+ and CD4+ T cell responses. For example, the intracellular cytokine assay combined with flow cytometry can be used (see, e.g., Suni M.A. et al., J Immunol Methods. 1998; 212:89-98; Nomura L.E. et al., Cytometry. 2000; 40:60-68; and Ghanekar S.A. et al., Clinical and Diagnostic Laboratory Immunology. 2001; 8:628-63). Briefly, the assay comprises the following steps: activation of cells via specific peptides or protein, an inhibition of protein transport (e.g., brefeldin A) is added to retain the cytokines within the cell. After washing, antibodies to other cellular markers can be added to the cells. Cells are then fixed and permeabilized. The anti-cytokine antibody is added and the cells can be analyzed by flow cytometry.

[0386] Assay for Confirming Replication-Deficiency of Viral Vectors Any assay known to the skilled artisan that determines concentration of infectious and replicationcompetent virus particles can also be used as a to measure replication-deficient viral particles in a sample. For example, FFU assays (as described in [00408]) with non-complementing cells can be used for this purpose.

[0387] Furthermore, plaque-based assays are the standard method used to determine virus concentration in terms of plaque forming units (PFU) in a virus sample. Specifically, a confluent monolayer of non-complementing host cells is infected with the virus at varying dilutions and covered with a semi-solid medium, such as agar to prevent the virus infection from spreading indiscriminately. A viral plaque is formed when a virus successfully infects and replicates itself in a cell within the fixed cell monolayer (see, e.g., Kaufmann, S.H.; Kabelitz, D. (2002). Methods in Microbiology Vol.32:Immunology of Infection. Academic Press. ISBN 0-12-521532-0). Plaque formation can take 3-14

days, depending on the virus being analyzed. Plaques are generally counted manually and the results, in combination with the dilution factor used to prepare the plate, are used to calculate the number of plaque forming units per sample unit volume (PFU/mL). The PFU/mL result represents the number of infective replicationcompetent particles within the sample.

[0388] Measuring Viral Load in the Blood or Liver Any assay known to the skilled artisan that determines the viral load may be used to detect the number of HBV particles per volume in the blood or liver *(see, e.g.,* Mendy et al., 2010, J. Viral Hepat. 17(2): 115-122). Non-limiting examples of such assays include nucleic acid-based tests such as PCR, as well as nonnucleic acid-based tests.

[0389] Liver Biopsy Any procedure known to the skilled artisan that performs a liver biopsy may be used to determine the degree of liver damage, for example, to test a patient for chronic HBV infection or liver cancer. Non-limiting examples of types of liver biopsies include percutaneous needle biopsies, laparoscopic biopsies, and transvenous biopsies. In certain embodiments, a liver biopsy is used to determine the presence of ground glass hepatocytes when the cells are examined under a light microscope. The observance of ground glass hepatocytes is indicative of the presence of HBsAg in the liver cells.

[0390] Assay for Expression of Viral Antigen Any assay known to the skilled artisan can be used for measuring expression of viral antigens. For example, FFU assays (as described in [00408]) can be performed. For detection, mono- or polyclonal antibody preparation(s) against respective viral antigens are used (transgene-specific FFU).

[0391] Furthermore, Western Blotting (as described in [00415]) can be performed.

[0392] Microparticle Enzyme Immunoassay The AXSYM® HbsAg (Abbott) is a microparticle enzyme immunoassay (MEIA) to detect HBsAg in adult, pediatric, and neonatal serum or plasma, including in pregnant women. This assay can be used as an aid in the diagnosis of acute or chronic HBV. This assay may also be used to confirm the presence of HBV infection.

[0393] To perform the assay, a sample of the patient's blood is placed into reaction wells containing detector antibodies and microparticles coated with antibodies to HBV (*e.g.,* to HBV antigens). If the blood sample contains HBV proteins (*e.g.*, HBsAg), they will bind to the microparticles in the reaction wells. This reaction is detected by another substance that produces light, which is then measured to determine the presence of HBV (e.g. HBV antigens) in the blood. If the first test is positive, the patient's blood is re-tested to confirm the presence of HBV (*e.g.,* HBV antigens). Any microparticle enzyme immunoassay

known to the skilled artisan may be used to measure the presence of HBsAg or other HBV antigens.

[0394] Other HBV Assays A sample of the patient's blood is placed in contact with either HBV antibodies or HBV antigens. The antibodies and/or antigens include HBsAg, antibodies to HBeAg, antibodies to HBsAg, HBeAg, IgM antibodies to HBcAg, and antibodies to HBcAg. If the patient is infected with HBV, antigens and/or antibodies present in the blood will cause a chemical reaction to occur when the test is run. This assay allows for the detection of the stage of HBV, according to what HBV antigens and/or antibodies are present in the patient's blood.

[0395] Any assay known to one of skill in the art may be used to evaluate levels of HBV, HBV antigens, or HBV antibodies. For non-limiting examples of such assays, *see, e.g.,* Mayer et al., 2012, BMC Clin. Pathol. 12:8, Van Helden et al., 2004, Clin. Lab. 50(1-2):63-73, and Villar et al., 2011, J. Med. Virol. 83(9):1522-1529.,

[0396] Animal Models The safety, tolerance and immunogenic effectiveness of vaccines comprising of an infectious arenavirus expressing an HBV antigen described herein or a composition thereof can be tested in animals models. In certain embodiments, the animal models that can be used to test the safety, tolerance and immunogenic effectiveness of the vaccines and compositions thereof used herein include mouse, guinea pig, rat, monkey, and chimpanzee. In a preferred embodiment, the animal models that can be used to test the safety, tolerance and immunogenic effectiveness of the vaccines and compositions thereof used herein include mouse.

[0397] In a specific example, a transgenic mouse model may be used to assess the antiviral potential of pharmacological agents, such as immunotherapies or vaccines, and to assess physiological processes, including the immune response *(see, e.g.,* Guidotti et al., 1995, J. Virol. 69(10):6158-69). Such transgenic mouse models may express human molecules, such as human class I and II HLA molecules, and/or the hepatitis B surface antigen (HBsAg) (*see, e.g.,* Bourgine et al., 2012, Virology 430(1):10-9).

[0398] In another specific example, the woodchuck (*Marmota monax*) can be used as an animal model for developing and testing treatment and prevention approaches to chronic hepadnaviral infections, such as chronic hepatitis B *(see, e.g.,* Kosinska et al., Hepat. Res. Treat. 2010:817580). The woodchuck model is applicable for evaluation of the immunogenicity and other immune responses of potential immunotherapies such as vaccines *(see, e.g.,* Vaccine 27(25-26):3271-3275).

6.10 Sequences

[0399] The sequences in Table 3 are illustrative amino acid sequences and nucleotide sequences that can be used with the methods and compositions described herein. In some instances a DNA sequence is used to describe the RNA sequence of a viral genomic segment. The RNA sequence can be readily deduced from the DNA sequence.

Table 3. Illustrative amino acid sequences.

SEQ ID NO:	Description	Sequence
1	nucleotide sequence of the HBV pre-S2/S ORF	ATGCAGTGGAATTCCACAACCTTCCACCAAACTCT GCAAGATCCCAGAGTGAGAGGCCTGTATTTCCCT GCTGGTGGCTCCAGTTCAGGAACAGTCAACCCTG TTCTGACCACTGCCTCTCCCTTGTCATCAATCTTCT CCAGGATTGGGGACCCTGCTCTGAACATGGAGAA CATCACATCAGGATTCCTGGGACCCCTTCTTGTGT TGCAGGCAGGGTTTTTCTTGTTGACAAGAATCCTC ACAATCCCTCAGAGTCTGGACTCTTGGTGGACTTC TCTCAATTTTCTGGGGGGAACCACAGTGTGTCTTG GCCAAAATTCTCAGTCCCCAACCTCCAATCACTCA CCAACCTCTTGTCCTCCAACTTGTCCTGGTTACAG ATGGATGTGTCTGAGGAGATTCATCATCTTCCTCT TCATCCTGCTGCTGTGCCTCATCTTCTTGTTGGTTC TTCTGGACTATCAAGGAATGTTGCCAGTTTGTCCT CTGATTCCAGGATCCTCAACAACCAGCACTGGAC CATGCAGGACCTGCATGACCACTGCTCAAGGAAC CTCAATGTATCCCTCCTGTTGCTGCACCAAACCTT
		CAGATGGAAATTGCACCTGCATTCCCATCCCATCA TCCTGGGCTTTTGGAAAATTCCTTTGGGAGTGGGC CTCAGCCAGATTCTCCTGGCTCAGTTTGCTGGTGC CATTTGTTCAGTGGTTTGTTGGGCTTTCCCCCACT GTTTGGCTTTCAGTGATTTGGATGATGTGGTATTG GGGGCCAAGTCTGTACAGCATCTTGAGTCCCTTTT TGCCTCTGTTGCCAATTTTCTTTTGTCTTTGGGTCT ACATTTAA

SEQ ID NO:	Description	Sequence
2	nucleotide sequence of the HBV HBc ORF	ATGGACATTGACCCTTACAAAGAATTTGGAGCAA CTGTGGAGTTGCTCTCCTTTTTGCCTTCTGACTTCT TTCCTTCAGTGAGAGATCTTCTTGACACTGCCTCA GCTCTGTACAGGGAAGCCTTGGAGTCTCCTGAGC ATTGTTCACCTCACCACACTGCACTCAGGCAAGC AATTCTTTGCTGGGGGGAACTCATGACTCTGGCA ACCTGGGTGGGTGTCAATTTGGAAGATCCAGCCT CAAGAGACCTTGTGGTCAGTTATGTCAACACAAA CATGGGCCTGAAGTTCAGGCAACTCTTGTGGTTTC ACATTTCTTGTCTCACTTTTGGAAGAGAAACAGTC ATTGAGTATTTGGTGTCTTTTGGAGTGTGGATCAG GACTCCTCCAGCTTACAGACCACCAAATGCCCCA ATCCTGTCAACACTTCCAGAGACCACTGTTGTCAG AAGAAGAGGCAGGTCCCCCAGAAGAAGAACTCC CTCACCAAGAAGAAGAAGGTCTCAATCTCCCAGA AGGAGAAGATCTCAATCAAGGGAATCTCAATGTT AG
3	nucleotide sequence of the HBV HBs-HBc fusion protein ORF	ATGGGGCAGAATCTTTCCACCAGCAATCCTCTGGGATTCTT TCCAGACCACCAGTTGGATCCAGCCTTCAGAGCAAACACTG CAAATCCAGATTGGGACTTCAATCCCAACAAGGACACCTGG CCAGATGCCAACAAGGTGGGAGCTGGAGCATTTGGGCTGGG TTTCACCCCACCCCATGGAGGCCTTTTGGGGTGGAGCCCTC

SEQ ID NO:	Description	Sequence
		AGGCTCAGGGCATTCTGCAAACTTTGCCAGCAAATCCACCT CCTGCCTCCACCAACAGGCAGTCAGGAAGGCAGCCCACCCC TCTGTCTCCACCTTTGAGAAACACTCATCCTCAGGCCATGC AGTGGAATTCCACAACCTTCCACCAAACTCTGCAAGATCCC AGAGTGAGAGGCCTGTATTTCCCTGCTGGTGGCTCCAGTTC AGGAACAGTCAACCCTGTTCTGACCACTGCCTCTCCCTTGT CATCAATCTTCTCCAGGATTGGGGACCCTGCTCTGAACATG GAGAACATCACATCAGGATTCCTGGGACCCCTTCTTGTGTT GCAGGCAGGGTTTTTCTTGTTGACAAGAATCCTCACAATCC CTCAGAGTCTGGACTCTTGGTGGACTTCTCTCAATTTTCTG GGGGGAACCACAGTGTGTCTTGGCCAAAATTCTCAGTCCCC AACCTCCAATCACTCACCAACCTCTTGTCCTCCAACTTGTC CTGGTTACAGATGGATGTGTCTGAGGAGATTCATCATCTTC CTCTTCATCCTGCTGCTGTGCCTCATCTTCTTGTTGGTTCT TCTGGACTATCAAGGAATGTTGCCAGTTTGTCCTCTGATTC CAGGATCCTCAACAACCAGCACTGGACCATGCAGGACCTGC ATGACCACTGCTCAAGGAACCTCAATGTATCCCTCCTGTTG CTGCACCAAACCTTCAGATGGAAATTGCACCTGCATTCCCA TCCCATCATCCTGGGCTTTTGGAAAATTCCTTTGGGAGTGG GCCTCAGCCAGATTCTCCTGGCTCAGTTTGCTGGTGCCATT TGTTCAGTGGTTTGTTGGGCTTTCCCCCACTGTTTGGCTTT CAGTGATTTGGATGATGTGGTATTGGGGGCCAAGTCTGTAC AGCATCTTGAGTCCCTTTTTGCCTCTGTTGCCAATTTTCTT TTGTCTTTGGGTCTACATTATGGACATTGACCCTTACAAAG AATTTGGAGCAACTGTGGAGTTGCTCTCCTTTTTGCCTTCT GACTTCTTTCCTTCAGTGAGAGATCTTCTTGACACTGCCTC AGCTCTGTACAGGGAAGCCTTGGAGTCTCCTGAGCATTGTT CACCTCACCACACTGCACTCAGGCAAGCAATTCTTTGCTGG GGGGAACTCATGACTCTGGCAACCTGGGTGGGTGTCAATTT GGAAGATCCAGCCTCAAGAGACCTTGTGGTCAGTTATGTCA ACACAAACATGGGCCTGAAGTTCAGGCAACTCTTGTGGTTT
		CACATTTCTTGTCTCACTTTTGGAAGAGAAACAGTCATTGA GTATTTGGTGTCTTTTGGAGTGTGGATCAGGACTCCTCCAG CTTACAGACCACCAAATGCCCCAATCCTGTCAACACTTCCA GAGACCACTGTTGTCAGAAGAAGAGGCAGGTCCCCCAGAAG AAGAACTCCCTCACCAAGAAGAAGAAGGTCTCAATCTCCCA GAAGGAGAAGATCTCAATCAAGGGAATCTCAATGTTAG

SEQ ID NO:	Description	Sequence
4	nucleotide sequence of the LCMV S segment expressing HBV HBs-HBc fusion protein in cDNA form (The genomic segment is RNA, the sequence in SEQ ID NO:4 is shown for DNA; however, exchanging all thymidines ("T") in SEQ ID NO:4 for uridines ("U") provides the RNA sequence.)	GCGCACCGGGGATCCTAGGCTTTTTGGATTGCGCT TTCCTCTAGATCAACTGGGTGTCAGGCCCTATCCT ACAGAAGGATGGGGCAGAATCTTTCCACCAGCAA TCCTCTGGGATTCTTTCCAGACCACCAGTTGGATC CAGCCTTCAGAGCAAACACTGCAAATCCAGATTG GGACTTCAATCCCAACAAGGACACCTGGCCAGAT GCCAACAAGGTGGGAGCTGGAGCATTTGGGCTGG GTTTCACCCCACCCCATGGAGGCCTTTTGGGGTGG AGCCCTCAGGCTCAGGGCATTCTGCAAACTTTGCC AGCAAATCCACCTCCTGCCTCCACCAACAGGCAG TCAGGAAGGCAGCCCACCCCTCTGTCTCCACCTTT GAGAAACACTCATCCTCAGGCCATGCAGTGGAAT TCCACAACCTTCCACCAAACTCTGCAAGATCCCA GAGTGAGAGGCCTGTATTTCCCTGCTGGTGGCTCC AGTTCAGGAACAGTCAACCCTGTTCTGACCACTG CCTCTCCCTTGTCATCAATCTTCTCCAGGATTGGG GACCCTGCTCTGAACATGGAGAACATCACATCAG GATTCCTGGGACCCCTTCTTGTGTTGCAGGCAGGG TTTTTCTTGTTGACAAGAATCCTCACAATCCCTCA GAGTCTGGACTCTTGGTGGACTTCTCTCAATTTTC TGGGGGGAACCACAGTGTGTCTTGGCCAAAATTC TCAGTCCCCAACCTCCAATCACTCACCAACCTCTT GTCCTCCAACTTGTCCTGGTTACAGATGGATGTGT CTGAGGAGATTCATCATCTTCCTCTTCATCCTGCT

SEQ ID NO:	Description	Sequence
		GCTGTGCCTCATCTTCTTGTTGGTTCTTCTGGACTA TCAAGGAATGTTGCCAGTTTGTCCTCTGATTCCAG GATCCTCAACAACCAGCACTGGACCATGCAGGAC CTGCATGACCACTGCTCAAGGAACCTCAATGTAT CCCTCCTGTTGCTGCACCAAACCTTCAGATGGAAA TTGCACCTGCATTCCCATCCCATCATCCTGGGCTT TTGGAAAATTCCTTTGGGAGTGGGCCTCAGCCAG ATTCTCCTGGCTCAGTTTGCTGGTGCCATTTGTTC AGTGGTTTGTTGGGCTTTCCCCCACTGTTTGGCTT TCAGTGATTTGGATGATGTGGTATTGGGGGCCAA GTCTGTACAGCATCTTGAGTCCCTTTTTGCCTCTG TTGCCAATTTTCTTTTGTCTTTGGGTCTACATTATG GACATTGACCCTTACAAAGAATTTGGAGCAACTG TGGAGTTGCTCTCCTTTTTGCCTTCTGACTTCTTTC CTTCAGTGAGAGATCTTCTTGACACTGCCTCAGCT CTGTACAGGGAAGCCTTGGAGTCTCCTGAGCATT GTTCACCTCACCACACTGCACTCAGGCAAGCAAT TCTTTGCTGGGGGGAACTCATGACTCTGGCAACCT GGGTGGGTGTCAATTTGGAAGATCCAGCCTCAAG AGACCTTGTGGTCAGTTATGTCAACACAAACATG GGCCTGAAGTTCAGGCAACTCTTGTGGTTTCACAT TTCTTGTCTCACTTTTGGAAGAGAAACAGTCATTG AGTATTTGGTGTCTTTTGGAGTGTGGATCAGGACT CCTCCAGCTTACAGACCACCAAATGCCCCAATCCT GTCAACACTTCCAGAGACCACTGTTGTCAGAAGA AGAGGCAGGTCCCCCAGAAGAAGAACTCCCTCAC CAAGAAGAAGAAGGTCTCAATCTCCCAGAAGGAG AAGATCTCAATCAAGGGAATCTCAATGTTAGAGA ACAGCGCCTCCCTGACTCTCCACCTCGAAAGAGG TGGAGAGTCAGGGAGGCCCAGAGGGTCTTAGAGT GTCACAACATTTGGGCCTCTAAAAATTAGGTCAT

SEQ ID NO:	Description	Sequence
		GTGGCAGAATGTTGTGAACAGTTTTCAGATCTGG GAGCCTTGCTTTGGAGGCGCTTTCAAAAATGATG CAGTCCATGAGTGCACAGTGCGGGGTGATCTCTTT CTTCTTTTTGTCCCTTACTATTCCAGTATGCATCTT ACACAACCAGCCATATTTGTCCCACACTTTATCTT CATACTCCCTCGAAGCTTCCCTGGTCATTTCAACA TCGATAAGCTTAATGTCCTTCCTATTTTGTGAGTC CAGAAGCTTTCTGATGTCATCGGAGCCTTGACAG CTTAGAACCATCCCCTGCGGAAGAGCACCTATAA CTGACGAGGTCAACCCGGGTTGCGCATTGAAGAG GTCGGCAAGATCCATGCCGTGTGAGTACTTGGAA TCTTGCTTGAATTGTTTTTGATCAACGGGTTCCCT GTAAAAGTGTATGAACTGCCCGTTCTGTGGTTGG AAAATTGCTATTTCCACTGGATCATTAAATCTACC CTCAATGTCAATCCATGTAGGAGCGTTGGGGTCA ATTCCTCCCATGAGGTCTTTTAAAAGCATTGTCTG GCTGTAGCTTAAGCCCACCTGAGGTGGACCTGCT GCTCCAGGCGCTGGCCTGGGTGAGTTGACTGCAG GTTTCTCGCTTGTGAGATCAATTGTTGTGTTTTCCC ATGCTCTCCCCACAATCGATGTTCTACAAGCTATG TATGGCCATCCTTCACCTGAAAGGCAAACTTTATA GAGGATGTTTTCATAAGGGTTCCTGTCCCCAACTT GGTCTGAAACAAACATGTTGAGTTTTCTCTTGGCC CCGAGAACTGCCTTCAAGAGATCCTCGCTGTTGCT TGGCTTGATCAAAATTGACTCTAACATGTTACCCC CATCCAACAGGGCTGCCCCTGCCTTCACGGCAGC ACCAAGACTAAAGTTATAGCCAGAAATGTTGATG CTGGACTGCTGTTCAGTGATGACCCCCAGAACTG GGTGCTTGTCTTTCAGCCTTTCAAGATCATTAAGA TTTGGATACTTGACTGTGTAAAGCAAGCCAAGGT CTGTGAGCGCTTGTACAACGTCATTGAGCGGAGT

SEQ ID NO:	Description	Sequence
		CTGTGACTGTTTGGCCATACAAGCCATAGTTAGAC TTGGCATTGTGCCAAATTGATTGTTCAAAAGTGAT GAGTCTTTCACATCCCAAACTCTTACCACACCACT TGCACCCTGCTGAGGCTTTCTCATCCCAACTATCT GTAGGATCTGAGATCTTTGGTCTAGTTGCTGTGTT GTTAAGTTCCCCATATATACCCCTGAAGCCTGGGG CCTTTCAGACCTCATGATCTTGGCCTTCAGCTTCT CAAGGTCAGCCGCAAGAGACATCAGTTCTTCTGC ACTGAGCCTCCCCACTTTCAAAACATTCTTCTTTG ATGTTGACTTTAAATCCACAAGAGAATGTACAGT CTGGTTGAGACTTCTGAGTCTCTGTAGGTCTTTGT CATCTCTCTTTTCCTTCCTCATGATCCTCTGAACAT TGCTGACCTCAGAGAAGTCCAACCCATTCAGAAG GTTGGTTGCATCCTTAATGACAGCAGCCTTCACAT CTGATGTGAAGCTCTGCAATTCTCTTCTCAATGCT TGCGTCCATTGGAAGCTCTTAACTTCCTTAGACAA GGACATCTTGTTGCTCAATGGTTTCTCAAGACAAA TGCGCAATCAAATGCCTAGGATCCACTGTGCG
5	nucleotide sequence of the LCMV S segment expressing the HBc ORF, in cDNA form (The genomic segment is RNA, the sequence in SEQ ID NO:5 is shown for DNA; however, exchanging all thymidines ("T") in SEQ ID NO:5 for uridines ("U") provides	GCGCACCGGGGATCCTAGGCTTTTTGGATTGCGCTTTCCTC TAGATCAACTGGGTGTCAGGCCCTATCCTACAGAAGGATGG ACATTGACCCTTACAAAGAATTTGGAGCAACTGTGGAGTTG CTCTCCTTTTTGCCTTCTGACTTCTTTCCTTCAGTGAGAGA TCTTCTTGACACTGCCTCAGCTCTGTACAGGGAAGCCTTGG AGTCTCCTGAGCATTGTTCACCTCACCACACTGCACTCAGG CAAGCAATTCTTTGCTGGGGGGAACTCATGACTCTGGCAAC CTGGGTGGGTGTCAATTTGGAAGATCCAGCCTCAAGAGACC TTGTGGTCAGTTATGTCAACACAAACATGGGCCTGAAGTTC AGGCAACTCTTGTGGTTTCACATTTCTTGTCTCACTTTTGG AAGAGAAACAGTCATTGAGTATTTGGTGTCTTTTGGAGTGT GGATCAGGACTCCTCCAGCTTACAGACCACCAAATGCCCCA ATCCTGTCAACACTTCCAGAGACCACTGTTGTCAGAAGAAG

SEQ ID NO:	Description	Sequence
	the RNA sequence.)	AGGCAGGTCCCCCAGAAGAAGAACTCCCTCACCAAGAAGAA GAAGGTCTCAATCTCCCAGAAGGAGAAGATCTCAATCAAGG GAATCTCAATGTTAGAGAACAGCGCCTCCCTGACTCTCCAC CTCGAAAGAGGTGGAGAGTCAGGGAGGCCCAGAGGGTCTTA GAGTGTCACAACATTTGGGCCTCTAAAAATTAGGTCATGTG GCAGAATGTTGTGAACAGTTTTCAGATCTGGGAGCCTTGCT TTGGAGGCGCTTTCAAAAATGATGCAGTCCATGAGTGCACA GTGCGGGGTGATCTCTTTCTTCTTTTTGTCCCTTACTATTC CAGTATGCATCTTACACAACCAGCCATATTTGTCCCACACT TTATCTTCATACTCCCTCGAAGCTTCCCTGGTCATTTCAAC ATCGATAAGCTTAATGTCCTTCCTATTTTGTGAGTCCAGAA GCTTTCTGATGTCATCGGAGCCTTGACAGCTTAGAACCATC CCCTGCGGAAGAGCACCTATAACTGACGAGGTCAACCCGGG TTGCGCATTGAAGAGGTCGGCAAGATCCATGCCGTGTGAGT ACTTGGAATCTTGCTTGAATTGTTTTTGATCAACGGGTTCC CTGTAAAAGTGTATGAACTGCCCGTTCTGTGGTTGGAAAAT TGCTATTTCCACTGGATCATTAAATCTACCCTCAATGTCAA TCCATGTAGGAGCGTTGGGGTCAATTCCTCCCATGAGGTCT TTTAAAAGCATTGTCTGGCTGTAGCTTAAGCCCACCTGAGG TGGACCTGCTGCTCCAGGCGCTGGCCTGGGTGAGTTGACTG CAGGTTTCTCGCTTGTGAGATCAATTGTTGTGTTTTCCCAT GCTCTCCCCACAATCGATGTTCTACAAGCTATGTATGGCCA TCCTTCACCTGAAAGGCAAACTTTATAGAGGATGTTTTCAT AAGGGTTCCTGTCCCCAACTTGGTCTGAAACAAACATGTTG AGTTTTCTCTTGGCCCCGAGAACTGCCTTCAAGAGATCCTC GCTGTTGCTTGGCTTGATCAAAATTGACTCTAACATGTTAC CCCCATCCAACAGGGCTGCCCCTGCCTTCACGGCAGCACCA AGACTAAAGTTATAGCCAGAAATGTTGATGCTGGACTGCTG TTCAGTGATGACCCCCAGAACTGGGTGCTTGTCTTTCAGCC TTTCAAGATCATTAAGATTTGGATACTTGACTGTGTAAAGC AAGCCAAGGTCTGTGAGCGCTTGTACAACGTCATTGAGCGG

SEQ ID NO:	Description	Sequence
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6	nucleotide sequence of the LCMV S segment expressing the pre-S2/S ORF, in cDNA form (The genomic segment is RNA, the sequence in SEQ ID NO:6 is shown for DNA; however, exchanging all thymidines ("T") in SEQ ID NO:6 for uridines ("U") provides the RNA sequence.)	GCGCACCGGGGATCCTAGGCTTTTTGGATTGCGCT TTCCTCTAGATCAACTGGGTGTCAGGCCCTATCCT ACAGAAGGATGCAGTGGAATTCCACAACCTTCCA CCAAACTCTGCAAGATCCCAGAGTGAGAGGCCTG TATTTCCCTGCTGGTGGCTCCAGTTCAGGAACAGT CAACCCTGTTCTGACCACTGCCTCTCCCTTGTCAT CAATCTTCTCCAGGATTGGGGACCCTGCTCTGAAC ATGGAGAACATCACATCAGGATTCCTGGGACCCC TTCTTGTGTTGCAGGCAGGGTTTTTCTTGTTGACA AGAATCCTCACAATCCCTCAGAGTCTGGACTCTTG GTGGACTTCTCTCAATTTTCTGGGGGGAACCACAG TGTGTCTTGGCCAAAATTCTCAGTCCCCAACCTCC AATCACTCACCAACCTCTTGTCCTCCAACTTGTCC TGGTTACAGATGGATGTGTCTGAGGAGATTCATC ATCTTCCTCTTCATCCTGCTGCTGTGCCTCATCTTC

SEQ ID NO:	Description	Sequence
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SEQ ID NO:	Description	Sequence
		TGAGGTGGACCTGCTGCTCCAGGCGCTGGCCTGG GTGAGTTGACTGCAGGTTTCTCGCTTGTGAGATCA ATTGTTGTGTTTTCCCATGCTCTCCCCACAATCGA TGTTCTACAAGCTATGTATGGCCATCCTTCACCTG AAAGGCAAACTTTATAGAGGATGTTTTCATAAGG GTTCCTGTCCCCAACTTGGTCTGAAACAAACATGT TGAGTTTTCTCTTGGCCCCGAGAACTGCCTTCAAG AGATCCTCGCTGTTGCTTGGCTTGATCAAAATTGA CTCTAACATGTTACCCCCATCCAACAGGGCTGCCC CTGCCTTCACGGCAGCACCAAGACTAAAGTTATA GCCAGAAATGTTGATGCTGGACTGCTGTTCAGTG ATGACCCCCAGAACTGGGTGCTTGTCTTTCAGCCT TTCAAGATCATTAAGATTTGGATACTTGACTGTGT AAAGCAAGCCAAGGTCTGTGAGCGCTTGTACAAC GTCATTGAGCGGAGTCTGTGACTGTTTGGCCATAC AAGCCATAGTTAGACTTGGCATTGTCCAAATTG ATTGTTCAAAAGTGATGAGTCTTTCACATCCCAAA CTCTTACCACACCACTTGCACCCTGCTGAGGCTTT CTCATCCCAACTATCTGTAGGATCTGAGATCTTTG GTCTAGTTGCTGTGTTGTTAAGTTCCCCATATATA CCCCTGAAGCCTGGGGCCTTTCAGACCTCATGATC TTGGCCTTCAGCTTCTCAAGGTCAGCCGCAAGAG ACATCAGTTCTTCTGCACTGAGCCTCCCCACTTTC AAAACATTCTTCTTTGATGTTGACTTTAAATCCAC AAGAGAATGTACAGTCTGGTTGAGACTTCTGAGT CTCTGTAGGTCTTTGTCATCTCTCTTTTCCTTCCTC ATGATCCTCTGAACATTGCTGACCTCAGAGAAGT CCAACCCATTCAGAAGGTTGGTTGCATCCTTAATG ACAGCAGCCTTCACATCTGATGTGAAGCTCTGCA ATTCTCTTCTCAATGCTTGCGTCCATTGGAAGCTC TTAACTTCCTTAGACAAGGACATCTTGTTGCTCAA
		TGGTTTCTCAAGACAAATGCGCAATCAAATGCCT AGGATCCACTGTGCG

SEQ ID NO:	Description	Sequence
7	lymphocytic choriomeningitis virus clone 13 segment L, complete sequence (GenBank: DQ361066.1) (The genomic segment is RNA, the sequence in SEQ ID NO: 7 is shown for DNA; however, exchanging all thymidines ("T") in SEQ ID NO: 7 for uridines ("U") provides the RNA sequence.)	GCGCACCGGGGATCCTAGGCGTTTAGTTGCGCTG TTTGGTTGCACAACTTTCTTCGTGAGGCTGTCAGA AGTGGACCTGGCTGATAGCGATGGGTCAAGGCAA GTCCAGAGAGGAGAAAGGCACCAATAGTACAAA CAGGGCCGAAATCCTACCAGATACCACCTATCTT GGCCCTTTAAGCTGCAAATCTTGCTGGCAGAAATT TGACAGCTTGGTAAGATGCCATGACCACTACCTTT GCAGGCACTGTTTAAACCTTCTGCTGTCAGTATCC GACAGGTGTCCTCTTTGTAAATATCCATTACCAAC CAGATTGAAGATATCAACAGCCCCAAGCTCTCCA CCTCCCTACGAAGAGTAACACCGTCCGGCCCCGG CCCCGACAAACAGCCCAGCACAAGGGAACCGCAC GTCaCCCAACGCACACAGACACAGCACCCAACAC AGAACACGCACACACACACACACACACCCACA CGCACGCGCCCCCACCACCGGGGGGCGCCCCCCC CCGGGGGGCGGCCCCCCGGGAGCCCGGGCGGAG CCCCACGGAGATGCCCATCAGTCGATGTCCTCGG CCACCGACCCGCCcAGCCAATCGTCGCAGGACCTC CCCTTGAGTCTAAACCTGCCCCCCACTgTTTCATA CATCAAAGTGCTCCTAGATTTGCTAAAACAAAGT CTGCAATCCTTAAAGGCGAACCAGTCTGGCAAAA GCGACAGTGGAATCAGCAGAATAGATCTGTCTAT ACATAGTTCCTGGAGGATTACACTTATCTCTGAAC CCAACAAATGTTCACCAGTTCTGAATCGATGCAG GAAGAGGTTCCCAAGGACATCACTAATCTTTTCAT AGCCCTCAAGTCCTGCTAGAAAGACTTTCATGTCC TTGGTCTCCAGCTTCACAATGATATTTTGGACAAG GTTTCTTCCTTCAAAAAGGGCACCCATCTTTACAG

SEQ ID NO:	Description	Sequence
		TCAGTGGCACAGGCTCCCACTCAGGTCCAACTCTC TCAAAGTCAATAGATCTAATCCCATCCAGTATTCT TTTGGAGCCCAACAACTCAAGCTCAAGAGAATCA CCAAGTATCAAGGGATCTTCCATGTAATCCTCAA ACTCTTCAGATCTGATATCAAAGACACCATCGTTC ACCTTGAAGACAGAGTCTGTCCTCAGTAAGTGGA GGCATTCATCCAACATTCTTCTATCTATCTCACCC TTAAAGAGGTGAGAGCATGATAAAAGTTCAGCCA CACCTGGATTCTGTAATTGGCACCTAACCAAGAA TATCAATGAAAATTTCCTTAAACAGTCAGTATTAT TCTGATTGTGCGTAAAGTCCACTGAAATTGAAAA CTCCAATACCCCTTTTGTGTAGTTGAGCATGTAGT CCCACAGATCCTTTAAGGATTTAAATGCCTTTGGG TTTGTCAGGCCCTGCCTAATCAACATGGCAGCATT ACACACAACATCTCCCATTCGGTAAGAGAACCAC CCAAAACCAAACTGCAAATCATTCCTAAACATAG GCCTCTCCACATTTTTGTTCACCACCTTTGAGACA AATGATTGAAAGGGGCCCAGTGCCTCAGCACCAT CTTCAGATGGCATCATTTCTTTATGAGGGAACCAT GAAAAATTGCCTAATGTCCTGGTTGTTGCAACAA ATTCTCGAACAAATGATTCAAAATACACCTGTTTT AAGAAGTTCTTGCAGACATCCCTCGTGCTAACAA CAAATTCATCAACCAGACTGGAGTCAGATCGCTG ATGAGAATTGGCAAGGTCAGAAAACAGAACAGT GTAATGTTCATCCCTTTTCCACTTAACAACATGAG AAATGAGTGACAAGGATTCTGAGTTAATATCAAT TAAAACACAGAGGTCAAGGAATTTAATTCTGGGA CTCCACCTCATGTTTTTTGAGCTCATGTCAGACAT AAATGGAAGAAGCTGATCCTCAAAGATCTTGGGA TATAGCCGCCTCACAGATTGAATCACTTGGTTCAA ATTCACTTTGTCCTCCAGTAGCCTTGAGCTCTCAG

SEQ ID NO:	Description	Sequence
		GCTTTCTTGCTACATAATCACATGGGTTTAAGTGC TTAAGAGTTAGGTTCTCACTGTTATTCTTCCCTTTG GTCGGTTCTGCTAGGACCCAAACACCCAACTCAA AAGAGTTGCTCAATGAAATACAAATGTAGTCCCA AAGAAGAGGCCTTAAAAGGCATATATGATCACGG TGGGCTTCTGGATGAGACTGTTTGTCACAAATGTA CAGCGTTATACCATCCCGATTGCAAACTCTTGTCA CATGATCATCTGTGGTTAGATCCTCAAGCAGCTTT TTGATATACAGATTTTCCCTATTTTTGTTTCTCACA CACCTGCTTCCTAGAGTTTTGCAAAGGCCTATAAA GCCAGATGAGATACAACTCTGGAAAGCTGACTTG TTGATTGCTTCTGACAGCAGCTTCTGTGCACCCCT TGTGAATTTACTACAAAGTTTGTTCTGGAGTGTCT TGATCAATGATGGGATTCTTTCCTCTTGGAAAGTC ATCACTGATGGATAAACCACCTTTTGTCTTAAAAC CATCCTTAATGGGAACATTTCATTCAAATTCAACC AGTTAACATCTGCTAACTGATTCAGATCTTCTTCA AGACCGAGGAGGTCTCCCAATTGAAGAATGGCCT CCTTTTATCTCTGTTAAATAGGTCTAAGAAAAATT CTTCATTAAATTCACCATTTTTGAGCTTATGATGC AGTTTCCTTACAAGCTTTCTTACAACCTTTGTTTCA TTAGGACACAGTTCCTCAATGAGTCTTTGTATTCT GTAACCTCTAGAACCATCCAGCCAATCTTTCACAT CAGTGTTGGTATTCAGTAGAAATGGATCCAAAGG GAAATTGGCATACTTTAGGAGGTCCAGTGTTCTCC TTTGGATACTATTAACTAGGGAGACTGGGACGCC ATTTGCGATGGCTTGATCTGCAATTGTATCTATTG TTTCACAAAGTTGATGTGGCTCTTTACACTTGACA TTGTGTAGCGCTGCAGATACAAACTTTGTGAGAA GAGGGACTTCCTCCCCCCATACATAGAATCTAGA TTTAAATTCTGCAGCGAACCTCCCAGCCACACTTT

SEQ ID NO:	Description	Sequence
		TTGGGCTGATAAATTTGTTTAACAAGCCGCTCAGA TGAGATTGGAATTCCAACAGGACAAGGACTTCCT CCGGATCACTTACAACCAGGTCACTCAGCCTCCTA TCAAATAAAGTGATCTGATCATCACTTGATGTGTA AGCCTCTGGTCTTTCGCCAAAGATAACACCAATG CAGTAGTTGATGAACCTCTCGCTAAGCAAACCAT AGAAGTCAGAAGCATTATGCAAGATTCCCTGCCC CATATCAATAAGGCTGGATATATGGGATGGCACT ATCCCCATTTCAAAATATTGTCTGAAAATTCTCTC AGTAACAGTTGTTTCTGAACCCCTGAGAAGTTTTA GCTTCGACTTGACATATGATTTCATCATTGCATTC ACAACAGGAAAGGGGACCTCGACAAGCTTATGCA TGTGCCAAGTTAACAAAGTGCTAACATGATCTTTC CCGGAACGCACATACTGGTCATCACCTAGTTTGA GATTTTGTAGAAACATTAAGAACAAAAATGGGCA CATCATTGGTCCCCATTTGCTGTGATCCATACTAT AGTTTAAGAACCCTTCCCGCACATTGATAGTCATT GACAAGATTGCATTTTCAAATTCCTTATCATTGTT TAAACAGGAGCCTGAAAAGAAACTTGAAAAAGA CTCAAAATAATCTTCTATTAACCTTGTGAACATTT TTGTCCTCAAATCTCCAATATAGAGTTCTCTATTT CCCCCAACCTGCTCTTTATAAGATAGTGCAAATTT CAGCCTTCCAGAGTCAGGACCTACTGAGGTGTAT GATGTTGGTGATTCTTCTGAGTAGAAGCACAGATT TTTCAAAGCAGCACTCATACATTgTGTCAACGACA GAGCTTTACTAAGGGACTCAGAATTACTTTCCCTC TCACTGATTCTCACGTCTTCTTCCAGTTTGTCCCA GTCAAATTTGAAATTCAAGCCTTGCCTTTGCATAT GCCTGTATTTCCCTGAGTACGCATTTGCATTCATT TGCAACAGAATCATCTTCATGCAAGAAAACCAAT CATTCTCAGAAAAGAACTTTCTACAAAGGTTTTTT

SEQ ID NO:	Description	Sequence
		GCCATCTCATCGAGGCCACACTGATCTTTAATGAC TGAGGTGAAATACAAAGGTGACAGCTCTGTGGAA CCCTCAACAGCCTCACAGATAAATTTCATGTCATC ATTGGTTAGACATGATGGGTCAAAGTCTTCTACTA AATGGAAAGATATTTCTGACAAGATAACTTTTCTT AAGTGAGCCATCTTCCCTGTTAGAATAAGCTGTA AATGATGTAGTCCTTTTGTATTTGTAAGTTTTTCTC CATCTCCTTTGTCATTGGCCCTCCTACCTCTTCTGT ACCGTGCTATTGTGGTGTTGACCTTTTCTTCGAGA CTTTTGAAGAAGCTTGTCTCTTCTTCTCCATCAAA ACATATTTCTGCCAGGTTGTCTTCCGATCTCCCTG TCTCTTCTCCCTTGGAACCGATGACCAATCTAGAG ACTAACTTGGAAACTTTATATTCATAGTCTGAGTG GCTCAACTTATACTTTTGTTTTCTTACGAAACTCTC CGTAATTTGACTCACAGCACTAACAAGCAATTTGT TAAAGTCATATTCCAGAAGTCGTTCTCCATTTAGA TGCTTATTAACCACCACACTTTTGTTACTAGCAAG ATCTAATGCTGTCGCACATCCAGAGTTAGTCATGG GATCTAGGCTGTTTAGCTTCTTCTCTCCTTTGAAA ATTAAAGTGCCGTTGTTAAATGAAGACACCATTA GGCTAAAGGCTTCCAGATTAACACCTGGAGTTGT ATGCTGACAGTCAATTTCTTTACTAGTGAATCTCT TCATTTGCTCATAGAACACACATTCTTCCTCAGGA GTGATTGCTTCCTTGGGGTTGACAAAAAAACCAA ATTGACTTTTGGGCTCAAAGAACTTTTCAAAACAT TTTATCTGATCTGTTAGCCTGTCAGGGGTCTCCTT TGTGATCAAATGACACAGGTATGACACATTCAAC ATAAATTTAAATTTTGCACTCAACAACACCTTCTC ACCAGTACCAAAAATAGTTTTTATTAGGAATCTA AGCAGCTTATACACCACCTTCTCAGCAGGTGTGAT CAGATCCTCCCTCAACTTATCCATTAATGATGTAG

SEQ ID NO:	Description	Sequence
		ATGAAAAATCTGACACTATTGCCATCACCAAATA TCTGACACTCTGTACCTGCTTTTGATTTCTCTTTGT TGGGTTGGTGAGCATTAGCAACAATAGGGTCCTC AGTGCAACCTCAATGTCGGTGAGACAGTCTTTCA AATCAGGACATGATCTAATCCATGAAATCATGAT GTCTATCATATTGTATAAGACCTCATCTGAAAAAA TTGGTAAAAAGAACCTTTTAGGATCTGCATAGAA GGAAATTAAATGACCATCCGGGCCTTGTATGGAG TAGCACCTTGAAGATTCTCCAGTCTTCTGGTATAA TAGGTGGTATTCTTCAGAGTCCAGTTTTATTACTT GGCAAAACACTTCTTTGCATTCTACCACTTGATAT CTCACAGACCCTATTTGATTTTGCCTTAGTCTAGC AACTGAGCTAGTTTTCATACTGTTTGTTAAGGCCA GACAAACAGATGATAATCTTCTCAGGCTCTGTAT GTTCTTCAGCTGCTCTGTGCTGGGTTGGAAATTGT AATCTTCAAACTTCGTATAATACATTATCGGGTGA GCTCCAATTTTCATAAAGTTCTCAAATTCAGTGAA TGGTATGTGGCATTCTTGCTCAAGGTGTTCAGACA GTCCGTAATGCTCGAAACTCAGTCCCACCACTAA CAGGCATTTTTGAATTTTTGCAATGAACTCACTAA TAGATGCCCTAAACAATTCCTCAAAAGACACCTTT CTAAACACCTTTGACTTTTTTCTATTCCTCAAAAG TCTAATGAACTCCTCTTTAGTGCTGTGAAAGCTTA CCAGCCTATCATTCACACTACTATAGCAACAACCC ACCCAGTGTTTATCATTTTTTAACCCTTTGAATTTC GACTGTTTTATCAATGAGGAAAGACACAAAACAT CCAGATTTAACAACTGTCTCCTTCTAGTATTCAAC AGTTTCAAACTCTTGACTTTGTTTAACATAGAGAG GAGCCTCTCATATTCAGTGCTAGTCTCACTTCCCC TTTCGTGCCCATGGGTCTCTGCAGTTATGAATCTC ATCAAAGGACAGGATTCGACTGCCTCCCTGCTTA

SEQ ID NO:	Description	Sequence
		ATGTTAAGATATCATCACTATCAGCAAGGTTTTCA TAGAGCTCAGAGAATTCCTTGATCAAGCCTTCAG GGTTTACTTTCTGAAAGTTTCTCTTTAATTTCCCAC TTTCTAAATCTCTTCTAAACCTGCTGAAAAGAGAG TTTATTCCAAAAACCACATCATCACAGCTCATGTT GGGGTTGATGCCTTCGTGGCACATCCTCATAATTT CATCATTGTGAGTTGACCTCGCATCTTTCAGAATT TTCATAGAGTCCATACCGGAGCGCTTGTCGATAGT AGTCTTCAGGGACTCACAGAGTCTAAAATATTCA GACTCTTCAAAGACTTTCTCATTTTGGTTAGAATA CTCCAAAAGTTTGAATAAAAGGTCTCTAAATTTG AAGTTTGCCCACTCTGGCATAAAACTATTATCATA ATCACAACGACCATCTACTATTGGAACTAATGTG ACACCCGCAACAGCAAGGTCTTCCCTGATGCATG CCAATTTGTTAGTGTCCTCTATAAATTTCTTCTCA AAACTGGCTGGaGGCTCCTAACAAAACACTCAAG AAGAATGAGAGAATTGTCTATCAGCTTGTAACCA TCAGGAATGATAAGTGGTAGTCCTGGGCATACAA TTCCAGACTCCACCAAAATTGTTTCCACAGACTTA TCGTCGTGGTTGTGTGTGCAGCCACTCTTGTCTGC ACTGTCTATTTCAATGCAGCGTGACAGCAACTTGA GTCCCTCAATCAGAACCATTCTGGGTTCCCTTTGT CCCAGAAAGTTGAGTTTCTGCCTTGACAACCTCTC ATCCTGTTCTATATAGTTTAAACATAACTCTCTCA ATTCTGAGATGATTTCATCCATTGCGCATCAAAAA GCCTAGGATCCTCGGTGCG
8	amino acid sequence of an HBV HBs proteinderived epitope	VWLSVIWM
9	amino acid sequence of an HBV HBs proteinderived epitope	IPQSLDSWWTSL
10	amino acid sequence of an HBV HBc proteinderived epitope	MGLKFRQL

SEQ ID NO:	Description	Sequence
11	lymphocytic choriomeningitis virus segment S, complete sequence (The genomic segment is RNA, the sequence in SEQ ID NO: 11 is shown for DNA; however, exchanging all thymidines ("T") in SEQ ID NO:11 for uridines ("U") provides the RNA sequence.)	CGCACCGGGGATCCTAGGCTTTTTGGATTGCGCTTTCCTC TAGATCAACTGGGTGTCAGGCCCTATCCTACAGAAGGATG GGTCAGATTGTGACAATGTTTGAGGCTCTGCCTCACATCA TCGATGAGGTGATCAACATTGTCATTATTGTGCTTATCGT GATCACGGGTATCAAGGCTGTCTACAATTTTGCCACCTGT GGGATATTCGCATTGATCAGTTTCCTACTTCTGGCTGGCA GGTCCTGTGGCATGTACGGTCTTAAGGGACCCGACATTTA CAAAGGAGTTTACCAATTTAAGTCAGTGGAGTTTGATATG TCACATCTGAACCTGACCATGCCCAACGCATGTTCAGCCA ACAACTCCCACCATTACATCAGTATGGGGACTTCTGGACT AGAATTGACCTTCACCAATGATTCCATCATCAGTCACAAC TTTTGCAATCTGACCTCTGCCTTCAACAAAAAGACCTTTG ACCACACACTCATGAGTATAGTTTCGAGCCTACACCTCAG TATCAGAGGGAACTCCAACTATAAGGCAGTATCCTGCGAC TTCAACAATGGCATAACCATCCAATACAACTTGACATTCT CAGATCGACAAAGTGCTCAGAGCCAGTGTAGAACCTTCAG AGGTAGAGTCCTAGATATGTTTAGAACTGCCTTCGGGGGG AAATACATGAGGAGTGGCTGGGGCTGGACAGGCTCAGATG GCAAGACCACCTGGTGTAGCCAGACGAGTTACCAATACCT GATTATACAAAATAGAACCTGGGAAAACCACTGCACATAT GCAGGTCCTTTTGGGATGTCCAGGATTCTCCTTTCCCAAG AGAAGACTAAGTTCTTCACTAGGAGACTAGCGGGCACATT CACCTGGACTTTGTCAGACTCTTCAGGGGTGGAGAATCCA

SEQ ID NO:	Description	Sequence
		GGTGGTTATTGCCTGACCAAATGGATGATTCTTGCTGCAG AGCTTAAGTGTTTCGGGAACACAGCAGTTGCGAAATGCAA TGTAAATCATGATGCCGAATTCTGTGACATGCTGCGACTA ATTGACTACAACAAGGCTGCTTTGAGTAAGTTCAAAGAGG ACGTAGAATCTGCCTTGCACTTATTCAAAACAACAGTGAA TTCTTTGATTTCAGATCAACTACTGATGAGGAACCACTTG AGAGATCTGATGGGGGTGCCATATTGCAATTACTCAAAGT TTTGGTACCTAGAACATGCAAAGACCGGCGAAACTAGTGT CCCCAAGTGCTGGCTTGTCACCAATGGTTCTTACTTAAAT GAGACCCACTTCAGTGATCAAATCGAACAGGAAGCCGATA ACATGATTACAGAGATGTTGAGGAAGGATTACATAAAGAG GCAGGGGAGTACCCCCCTAGCATTGATGGACCTTCTGATG TTTTCCACATCTGCATATCTAGTCAGCATCTTCCTGCACC TTGTCAAAATACCAACACACAGGCACATAAAAGGTGGCTC ATGTCCAAAGCCACACCGATTAACCAACAAAGGAATTTGT AGTTGTGGTGCATTTAAGGTGCCTGGTGTAAAAACCGTCT GGAAAAGACGCTGAAGAACAGCGCCTCCCTGACTCTCCAC CTCGAAAGAGGTGGAGAGTCAGGGAGGCCCAGAGGGTCTT AGAGTGTCACAACATTTGGGCCTCTAAAAATTAGGTCATG TGGCAGAATGTTGTGAACAGTTTTCAGATCTGGGAGCCTT GCTTTGGAGGCGCTTTCAAAAATGATGCAGTCCATGAGTG CACAGTGCGGGGTGATCTCTTTCTTCTTTTTGTCCCTTAC TATTCCAGTATGCATCTTACACAACCAGCCATATTTGTCC CACACTTTGTCTTCATACTCCCTCGAAGCTTCCCTGGTCA TTTCAACATCGATAAGCTTAATGTCCTTCCTATTCTGTGA GTCCAGAAGCTTTCTGATGTCATCGGAGCCTTGACAGCTT AGAACCATCCCCTGCGGAAGAGCACCTATAACTGACGAGG TCAACCCGGGTTGCGCATTGAAGAGGTCGGCAAGATCCAT GCCGTGTGAGTACTTGGAATCTTGCTTGAATTGTTTTTGA TCAACGGGTTCCCTGTAAAAGTGTATGAACTGCCCGTTCT GTGGTTGGAAAATTGCTATTTCCACTGGATCATTAAATCT

SEQ ID NO:	Description	Sequence
		ACCCTCAATGTCAATCCATGTAGGAGCGTTGGGGTCAATT CCTCCCATGAGGTCTTTTAAAAGCATTGTCTGGCTGTAGC TTAAGCCCACCTGAGGTGGACCTGCTGCTCCAGGCGCTGG CCTGGGTGAATTGACTGCAGGTTTCTCGCTTGTGAGATCA ATTGTTGTGTTTTCCCATGCTCTCCCCACAATCGATGTTC TACAAGCTATGTATGGCCATCCTTCACCTGAAAGGCAAAC TTTATAGAGGATGTTTTCATAAGGGTTCCTGTCCCCAACT TGGTCTGAAACAAACATGTTGAGTTTTCTCTTGGCCCCGA GAACTGCCTTCAAGAGGTCCTCGCTGTTGCTTGGCTTGAT CAAAATTGACTCTAACATGTTACCCCCATCCAACAGGGCT GCCCCTGCCTTCACGGCAGCACCAAGACTAAAGTTATAGC CAGAAATGTTGATGCTGGACTGCTGTTCAGTGATGACCCC CAGAACTGGGTGCTTGTCTTTCAGCCTTTCAAGATCATTA AGATTTGGATACTTGACTGTGTAAAGCAAGCCAAGGTCTG TGAGCGCTTGTACAACGTCATTGAGCGGAGTCTGTGACTG TTTGGCCATACAAGCCATAGTTAGACTTGGCATTGTGCCA AATTGATTGTTCAAAAGTGATGAGTCTTTCACATCCCAAA CTCTTACCACACCACTTGCACCCTGCTGAGGCTTTCTCAT CCCAACTATCTGTAGGATCTGAGATCTTTGGTCTAGTTGC TGTGTTGTTAAGTTCCCCATATATACCCCTGAAGCCTGGG GCCTTTCAGACCTCATGATCTTGGCCTTCAGCTTCTCAAG GTCAGCCGCAAGAGACATCAGTTCTTCTGCACTGAGCCTC CCCACTTTCAAAACATTCTTCTTTGATGTTGACTTTAAAT CCACAAGAGAATGTACAGTCTGGTTGAGACTTCTGAGTCT CTGTAGGTCTTTGTCATCTCTCTTTTCCTTCCTCATGATC CTCTGAACATTGCTGACCTCAGAGAAGTCCAACCCATTCA GAAGGTTGGTTGCATCCTTAATGACAGCAGCCTTCACATC TGATGTGAAGCTCTGCAATTCTCTTCTCAATGCTTGCGTC CATTGGAAGCTCTTAACTTCCTTAGACAAGGACATCTTGT TGCTCAATGGTTTCTCAAGACAAATGCGCAATCAAATGCC TAGGATCCACTGTGCG

SEQ ID NO:	Description	Sequence
12	lymphocytic choriomeningitis virus clone 13 segment S, complete sequence (GenBank: DQ361065.2) (The genomic segment is RNA, the sequence in SEQ ID NO: 12 is shown for DNA; however, exchanging all thymidines ("T") in SEQ ID NO: 12 for uridines ("U") provides the RNA sequence.)	GCGCACCGGGGATCCTAGGCTTTTTGGATTGCGCTTTCCT CTAGATCAACTGGGTGTCAGGCCCTATCCTACAGAAGGAT GGGTCAGATTGTGACAATGTTTGAGGCTCTGCCTCACATC ATCGATGAGGTGATCAACATTGTCATTATTGTGCTTATCG TGATCACGGGTATCAAGGCTGTCTACAATTTTGCCACCTG TGGGATATTCGCATTGATCAGTTTCCTACTTCTGGCTGGC AGGTCCTGTGGCATGTACGGTCTTAAGGGACCCGACATTT ACAAAGGAGTTTACCAATTTAAGTCAGTGGAGTTTGATAT GTCACATCTGAACCTGACCATGCCCAACGCATGTTCAGCC AACAACTCCCACCATTACATCAGTATGGGGACTTCTGGAC TAGAATTGACCTTCACCAATGATTCCATCATCAGTCACAA CTTTTGCAATCTGACCTCTGCCTTCAACAAAAAGACCTTT GACCACACACTCATGAGTATAGTTTCGAGCCTACACCTCA GTATCAGAGGGAACTCCAACTATAAGGCAGTATCCTGCGA CTTCAACAATGGCATAACCATCCAATACAACTTGACATTC TCAGATGCACAAAGTGCTCAGAGCCAGTGTAGAACCTTCA GAGGTAGAGTCCTAGATATGTTTAGAACTGCCTTCGGGGG GAAATACATGAGGAGTGGCTGGGGCTGGACAGGCTCAGAT GGCAAGACCACCTGGTGTAGCCAGACGAGTTACCAATACC TGATTATACAAAATAGAACCTGGGAAAACCACTGCACATA TGCAGGTCCTTTTGGGATGTCCAGGATTCTCCTTTCCCAA GAGAAGACTAAGTTCCTCACTAGGAGACTAGCGGGCACAT TCACCTGGACTTTGTCAGACTCTTCAGGGGTGGAGAATCC AGGTGGTTATTGCCTGACCAAATGGATGATTCTTGCTGCA GAGCTTAAGTGTTTCGGGAACACAGCAGTTGCGAAATGCA ATGTAAATCATGATGAAGAATTCTGTGACATGCTGCGACT AATTGACTACAACAAGGCTGCTTTGAGTAAGTTCAAAGAG GACGTAGAATCTGCCTTGCACTTATTCAAAACAACAGTGA ATTCTTTGATTTCAGATCAACTACTGATGAGGAACCACTT GAGAGATCTGATGGGGGTGCCATATTGCAATTACTCAAAG TTTTGGTACCTAGAACATGCAAAGACCGGCGAAACTAGTG

SEQ ID NO:	Description	Sequence
		TCCCCAAGTGCTGGCTTGTCACCAATGGTTCTTACTTAAA TGAGACCCACTTCAGTGACCAAATCGAACAGGAAGCCGAT AACATGATTACAGAGATGTTGAGGAAGGATTACATAAAGA GGCAGGGGAGTACCCCCCTAGCATTGATGGACCTTCTGAT GTTTTCCACATCTGCATATCTAGTCAGCATCTTCCTGCAC CTTGTCAAAATACCAACACACAGGCACATAAAAGGTGGCT CATGTCCAAAGCCACACCGATTAACCAACAAAGGAATTTG TAGTTGTGGTGCATTTAAGGTGCCTGGTGTAAAAACCGTC TGGAAAAGACGCTGAAGAACAGCGCCTCCCTGACTCTCCA CCTCGAAAGAGGTGGAGAGTCAGGGAGGCCCAGAGGGTCT TAGAGTGTCACAACATTTGGGCCTCTAAAAATTAGGTCAT GTGGCAGAATGTTGTGAACAGTTTTCAGATCTGGGAGCCT TGCTTTGGAGGCGCTTTCAAAAATGATGCAGTCCATGAGT GCACAGTGCGGGGTGATCTCTTTCTTCTTTTTGTCCCTTA CTATTCCAGTATGCATCTTACACAACCAGCCATATTTGTC CCACACTTTGTCTTCATACTCCCTCGAAGCTTCCCTGGTC ATTTCAACATCGATAAGCTTAATGTCCTTCCTATTCTGTG AGTCCAGAAGCTTTCTGATGTCATCGGAGCCTTGACAGCT TAGAACCATCCCCTGCGGAAGAGCACCTATAACTGACGAG GTCAACCCGGGTTGCGCATTGAAGAGGTCGGCAAGATCCA TGCCGTGTGAGTACTTGGAATCTTGCTTGAATTGTTTTTG ATCAACGGGTTCCCTGTAAAAGTGTATGAACTGCCCGTTC TGTGGTTGGAAAATTGCTATTTCCACTGGATCATTAAATC TACCCTCAATGTCAATCCATGTAGGAGCGTTGGGGTCAAT TCCTCCCATGAGGTCTTTTAAAAGCATTGTCTGGCTGTAG CTTAAGCCCACCTGAGGTGGACCTGCTGCTCCAGGCGCTG GCCTGGGTGAATTGACTGCAGGTTTCTCGCTTGTGAGATC AATTGTTGTGTTTTCCCATGCTCTCCCCACAATCGATGTT CTACAAGCTATGTATGGCCATCCTTCACCTGAAAGGCAAA CTTTATAGAGGATGTTTTCATAAGGGTTCCTGTCCCCAAC TTGGTCTGAAACAAACATGTTGAGTTTTCTCTTGGCCCCG

SEQ ID NO:	Description	Sequence
		AGAACTGCCTTCAAGAGGTCCTCGCTGTTGCTTGGCTTGA TCAAAATTGACTCTAACATGTTACCCCCATCCAACAGGGC TGCCCCTGCCTTCACGGCAGCACCAAGACTAAAGTTATAG CCAGAAATGTTGATGCTGGACTGCTGTTCAGTGATGACCC CCAGAACTGGGTGCTTGTCTTTCAGCCTTTCAAGATCATT AAGATTTGGATACTTGACTGTGTAAAGCAAGCCAAGGTCT GTGAGCGCTTGTACAACGTCATTGAGCGGAGTCTGTGACT GTTTGGCCATACAAGCCATAGTTAGACTTGGCATTGTGCC AAATTGATTGTTCAAAAGTGATGAGTCTTTCACATCCCAA ACTCTTACCACACCACTTGCACCCTGCTGAGGCTTTCTCA TCCCAACTATCTGTAGGATCTGAGATCTTTGGTCTAGTTG CTGTGTTGTTAAGTTCCCCATATATACCCCTGAAGCCTGG GGCCTTTCAGACCTCATGATCTTGGCCTTCAGCTTCTCAA GGTCAGCCGCAAGAGACATCAGTTCTTCTGCACTGAGCCT CCCCACTTTCAAAACATTCTTCTTTGATGTTGACTTTAAA TCCACAAGAGAATGTACAGTCTGGTTGAGACTTCTGAGTC TCTGTAGGTCTTTGTCATCTCTCTTTTCCTTCCTCATGAT CCTCTGAACATTGCTGACCTCAGAGAAGTCCAACCCATTC AGAAGGTTGGTTGCATCCTTAATGACAGCAGCCTTCACAT CTGATGTGAAGCTCTGCAATTCTCTTCTCAATGCTTGCGT CCATTGGAAGCTCTTAACTTCCTTAGACAAGGACATCTTG TTGCTCAATGGTTTCTCAAGACAAATGCGCAATCAAATGC CTAGGATCCACTGTGCG
13	lymphocytic choriomeningitis strain MP segment L, complete sequence (The genomic segment is RNA, the sequence in SEQ ID NO:13 is shown for DNA;	GCGCACCGGGGATCCTAGGCATTTTTGTTGCGCATTTTGT TGTGTTATTTGTTGCACAGCCCTTCATCGTGGGACCTTCA CAAACAAACCAAACCACCAGCCATGGGCCAAGGCAAGTCC AAAGAGGGAAGGGATGCCAGCAATACGAGCAGAGCTGAAA TTCTGCCAGACACCACCTATCTCGGACCTCTGAACTGCAA GTCATGCTGGCAGAGATTTGACAGTTTAGTCAGATGCCAT GACCACTATCTCTGCAGACACTGCCTGAACCTCCTGCTGT CAGTCTCCGACAGGTGCCCTCTCTGCAAACATCCATTGCC

SEQ ID NO:	Description	Sequence
	however, exchanging all thymidines ("T") in SEQ ID NO:13 for uridines ("U") provides the RNA sequence.)	AACCAAACTGAAAATATCCACGGCCCCAAGCTCTCCACCC CCTTACGAGGAGTGACGCCCCGAGCCCCAACACCGACACA AGGAGGCCACCAACACAACGCCCAACACGGAACACACACA CACACACCCACACACACATCCACACACACGCGCCCCACA ACGGGGGCGCCCCCCCCGGGGGTGGCCCCCCGGGTGCTCGG GCGGAGCCCCACGGAGAGGCCAATTAGTCGATCTCCTCGA CCACCGACTTGGTCAGCCAGTCATCACAGGACTTGCCCTT AAGTCTGTACTTGCCCACAACTGTTTCATACATCACCGTG TTCTTTGACTTACTGAAACATAGCCTACAGTCTTTGAAAG TGAACCAGTCAGGCACAAGTGACAGCGGTACCAGTAGAAT GGATCTATCTATACACAACTCTTGGAGAATTGTGCTAATT TCCGACCCCTGTAGATGCTCACCAGTTCTGAATCGATGTA GAAGAAGGCTCCCAAGGACGTCATCAAAATTTCCATAACC CTCGAGCTCTGCCAAGAAAACTCTCATATCCTTGGTCTCC AGTTTCACAACGATGTTCTGAACAAGGCTTCTTCCCTCAA AAAGAGCACCCATTCTCACAGTCAAGGGCACAGGCTCCCA TTCAGGCCCAATCCTCTCAAAATCAAGGGATCTGATCCCG TCCAGTATTTTCCTTGAGCCTATCAGCTCAAGCTCAAGAG AGTCACCGAGTATCAGGGGGTCCTCCATATAGTCCTCAAA CTCTTCAGACCTAATGTCAAAAACACCATCGTTCACCTTG AAGATAGAGTCTGATCTCAACAGGTGGAGGCATTCGTCCA AGAACCTTCTGTCCACCTCACCTTTAAAGAGGTGAGAGCA TGATAGGAACTCAGCTACACCTGGACCTTGTAACTGGCAC TTCACTAAAAAGATCAATGAAAACTTCCTCAAACAATCAG TGTTATTCTGGTTGTGAGTGAAATCTACTGTAATTGAGAA CTCTAGCACTCCCTCTGTATTATTTATCATGTAATCCCAC AAGTTTCTCAAAGACTTGAATGCCTTTGGATTTGTCAAGC CTTGTTTGATTAGCATGGCAGCATTGCACACAATATCTCC CAATCGGTAAGAGAACCATCCAAATCCAAATTGCAAGTCA TTCCTAAACATGGGCCTCTCCATATTTTTGTTCACTACTT TTAAGATGAATGATTGCAAAGGCCCCAATGCTTCAGCGCC

SEQ ID NO:	Description	Sequence
		ATCTTCAGATGGCATCATGTCTTTATGAGGGAACCATGAA AAACTTCCTAGAGTTCTGCTTGTTGCTACAAATTCTCGTA CAAATGACTCAAAATACACTTGTTTTAAAAAGTTTTTGCA GACATCCCTTGTACTAACGACAAATTCATCAACAAGGCTT GAGTCAGAGCGCTGATGGGAATTTACAAGATCAGAAAATA GAACAGTGTAGTGTTCGTCCCTCTTCCACTTAACTACATG AGAAATGAGCGATAAAGATTCTGAATTGATATCGATCAAT ACGCAAAGGTCAAGGAATTTGATTCTGGGACTCCATCTCA TGTTTTTTGAGCTCATATCAGACATGAAGGGAAGCAGCTG ATCTTCATAGATTTTAGGGTACAATCGCCTCACAGATTGG ATTACATGGTTTAAACTTATCTTGTCCTCCAGTAGCCTTG AACTCTCAGGCTTCCTTGCTACATAATCACATGGGTTCAA GTGCTTGAGGCTTGAGCTTCCCTCATTCTTCCCTTTCACA GGTTCAGCTAAGACCCAAACACCCAACTCAAAGGAATTAC TCAGTGAGATGCAAATATAGTCCCAAAGGAGGGGCCTCAA GAGACTGATGTGGTCGCAGTGAGCTTCTGGATGACTTTGC CTGTCACAAATGTACAACATTATGCCATCATGTCTGTGGA TTGCTGTCACATGCGCATCCATAGCTAGATCCTCAAGCAC TTTTCTAATGTATAGATTGTCCCTATTTTIATTTCTCACA CATCTACTTCCCAAAGTTTTGCAAAGACCTATAAAGCCTG ATGAGATGCAACTTTGAAAGGCTGACTTATTGATTGCTTC TGACAGCAACTTCTGTGCACCTCTTGTGAACTTACTGCAG AGCTTGTTCTGGAGTGTCTTGATTAATGATGGATTCTTT CCTCTTGGAAAGTCATTACTGATGGATAAACCACTTTCTG CCTCAAGACCATTCTTAATGGGAACAACTCATTCAAATTC AGCCAATTTATGTTTGCCAATTGACTTAGATCCTCTTCGA GGCCAAGGATGTTTCCCAACTGAAGAATGGCTTCCTTTTT ATCCCTATTGAAGAGGTCTAAGAAGAATTCTTCATTGAAC TCACCATTCTTGAGCTTATGATGTAGTCTCCTTACAAGCC TTCTCATGACCTTCGTTTCACTAGGACACAATTCTTCAAT AAGCCTTTGGATTCTGTAACCTCTAGAGCCATCCAACCAA

SEQ ID NO:	Description	Sequence
		TCCTTGACATCAGTATTAGTGTTAAGCAAAAATGGGTCCA AGGGAAAGTTGGCATATTTTAAGAGGTCTAATGTTCTCTT CTGGATGCAGTTTACCAATGAAACTGGAACACCATTTGCA ACAGCTTGATCGGCAATTGTATCTATTGTTTCACAGAGTT GGTGTGGCTCTTTACACTTAACGTTGTGTAATGCTGCTGA CACAAATTTTGTTAAAAGTGGGACCTCTTCCCCCCACACA TAAAATCTGGATTTAAATTCTGCAGCAAATCGCCCCACCA CACTTTTCGGACTGATGAACTTGTTAAGCAAGCCACTCAA ATGAGAATGAAATTCCAGCAATACAAGGACTTCCTCAGGG TCACTATCAACCAGTTCACTCAATCTCCTATCAAATAAGG TGATCTGATCATCACTTGATGTGTAAGATTCTGGTCTCTC ACCAAAAATGACACCGATACAATAATTAATGAATCTCTCA CTGATTAAGCCGTAAAAGTCAGAGGCATTATGTAAGATTC CCTGTCCCATGTCAATGAGACTGCTTATATGGGAAGGCAC TATTCCTAATTCAAAATATTCTCGAAAGATTCTTTCAGTC ACAGTTGTCTCTGAACCCCTAAGAAGTTTCAGCTTTGATT TGATATATGATTTCATCATTGCATTCACAACAGGAAAAGG GACCTCAACAAGTTTGTGCATGTGCCAAGTTAATAAGGTG CTGATATGATCCTTTCCGGAACGCACATACTGGTCATCAC CCAGTTTGAGATTTTGAAGGAGCATTAAAAACAAAAATGG GCACATCATTGGCCCCCATTTGCTATGATCCATACTGTAG TTCAACAACCCCTCTCGCACATTGATGGTCATTGATAGAA TTGCATTTTCAAATTCTTTGTCATTGTTTAAGCATGAACC TGAGAAGAAGCTAGAAAAAGACTCAAAATAATCCTCTATC AATCTTGTAAACATTTTTGTTCTCAAATCCCCAATATAAA GTTCTCTGTTTCCTCCAACCTGCTCTTTGTATGATAACGC AAACTTCAACCTTCCGGAATCAGGACCAACTGAAGTGTAT GACGTTGGTGACTCCTCTGAGTAAAAACATAAATTCTTTA AAGCAGCACTCATGCATTTTGTCAATGATAGAGCCTTACT TAGAGACTCAGAATTACTTTCCCTTTCACTAATTCTAACA TCTTCTTCTAGTTTGTCCCAGTCAAACTTGAAATTCAGAC

SEQ ID NO:	Description	Sequence
		CTTGTCTTTGCATGTGCCTGTATTTCCCTGAGTATGCATT TGCATTCATTTGCAGTAGAATCATTTTCATACACGAAAAC CAATCACCCTCTGAAAAAAACTTCCTGCAGAGGTTTTTTG CCATTTCATCCAGACCACATTGTTCTTTGACAGCTGAAGT GAAATACAATGGTGACAGTTCTGTAGAAGTTTCAATAGCC TCACAGATAAATTTCATGTCATCATTGGTGAGACAAGATG GGTCAAAATCTTCCACAAGATGAAAAGAAATTTCTGATAA GATGACCTTCCTTAAATATGCCATTTTACCTGACAATATA GTCTGAAGGTGATGCAATCCTTTTGTATTTTCAAACCCCA CCTCATTTTCCCCTTCATTGGTCTTCTTGCTTCTTTCATA CCGCTTTATTGTGGAGTTGACCTTATCTTCTAAATTCTTG AAGAAACTTGTCTCTTCTTCCCCATCAAAGCATATGTCTG CTGAGTCACCTTCTAGTTTCCCAGCTTCTGTTTCTTTAGA GCCGATAACCAATCTAGAGACCAACTTTGAAACCTTGTAC TCGTAATCTGAGTGGTTCAATTTGTACTTCTGCTTTCTCA TGAAGCTCTCTGTGATCTGACTCACAGCACTAACAAGCAA TTTGTTAAAATCATACTCTAGGAGCCGTTCCCCATTTAAA TGTTTGTTAACAACCACACTTTTGTTGCTGGCAAGGTCTA ATGCTGTTGCACACCCAGAGTTAGTCATGGGATCCAAGCT ATTGAGCCTCTTCTCCCCTTTGAAAATCAAAGTGCCATTG TTGAATGAGGACACCATCATGCTAAAGGCCTCCAGATTGA CACCTGGGGTTGTGCGCTGACAGTCAACTTCTTTCCCAGT GAACTTCTTCATTTGGTCATAAAAAACACACTCTTCCTCA GGGGTGATTGACTCTTTAGGGTTAACAAAGAAGCCAAACT CACTTTTAGGCTCAAAGAATTTCTCAAAGCATTTAATTTG ATCTGTCAGCCTATCAGGGGTTTCCTTTGTGATTAAATGA CACAGGTATGACACATTCAACATGAACTTGAACTTTGCGC TCAACAGTACCTTTTCACCAGTCCCAAAAACAGTTTTGAT CAAAAATCTGAGCAATTTGTACACTACTTTCTCAGCAGGT GTGATCAAATCCTCCTTCAACTTGTCCATCAATGATGTGG ATGAGAAGTCTGAGACAATGGCCATCACTAAATACCTAAT

SEQ ID NO:	Description	Sequence
		GTTTTGAACCTGTTTTTGATTCCTCTTTGTTGGGTTGGTG AGCATGAGTAATAATAGGGTTCTCAATGCAATCTCAACAT CATCAATGCTGTCCTTCAAGTCAGGACATGATCTGATCCA TGAGATCATGGTGTCAATCATGTTGTGCAACACTTCATCT GAGAAGATTGGTAAAAAGAACCTTTTTGGGTCTGCATAAA AAGAGATTAGATGGCCATTGGGACCTTGTATAGAATAACA CCTTGAGGATTCTCCAGTCTTTTGATACAGCAGGTGATAT TCCTCAGAGTCCAATTTTATCACTTGGCAAAATACCTCTT TACATTCCACCACTTGATACCTTACAGAGCCCAATTGGTT TTGTCTTAATCTAGCAACTGAACTTGTTTTCATACTGTTT GTCAAAGCTAGACAGACAGATGACAATCTTTTCAAACTAT GCATGTTCCTTAATTGTTCCGTATTAGGCTGGAAATCATA ATCTTCAAACTTTGTATAATACATTATAGGATGAGTTCCG GACCTCATGAAATTCTCAAACTCAATAAATGGTATGTGGC ACTCATGCTCAAGATGTTCAGACAGACCATAGTGCCCAAA ACTAAGTCCCACCACTGACAAGCACCTTTGAACTTTTAAA ATGAACTCATTTATGGATGTTCTAAACAAATCCTCAAGAG ATACCTTTCTATACGCCTTTGACTTTCTCCTGTTCCTTAG AAGTCTGATGAACTCTTCCTTGGTGCTATGAAAGCTCACC AACCTATCATTCACACTCCCATAGCAACAACCTAACCCAGT GCTTATCATTTTTTGACCCTTTGAGTTTAGACTGTTTGAT CAACGAAGAGAGACACAAGACATCCAAATTCAGTAACTGT CTCCTTCTGGTGTTCAATAATTTTAAACTTTTAACTTTGT TCAACATAGAGAGGAGCCTCTCATACTCAGTGCTAGTCTC ACTTCCTCTCTCATAACCATGGGTATCTGCTGTGATAAAT CTCATCAAAGGACAGGATTCAACTGCCTCCTTGCTTAGTG CTGAAATGTCATCACTGTCAGCAAGAGTCTCATAAAGCTC AGAGAATTCCTTAATTAAATTTCCGGGGTTGATTTTCTGA AAACTCCTCTTGAGCTTCCCAGTTTCCAAGTCTCTTCTAA ACCTGCTGTAAAGGGAGTTTATGCCAAGAACCACATCATC GCAGTTCATGTTTGGGTTGACACCATCATGGCACATTTTC

SEQ ID NO:	Description	Sequence
		ATAATTTCATCATTGTGAAATGATCTTGCATCTTTCAAGA TTTTCATAGAGTCTATACCGGAACGCTTATCAACAGTGGT CTTGAGAGATTCGCAAAGTCTGAAGTACTCAGATTCCTCA AAGACTTTCTCATCTTGGCTAGAATACTCTAAAAGTTTAA ACAGAAGGTCTCTGAACTTGAAATTCACCCACTCTGGCAT AAAGCTGTTATCATAATCACACCGACCATCCACTATTGGG ACCAATGTGATACCCGCAATGGCAAGGTCTTCTTTGATAC AGGCTAGTTTATTGGTGTCCTCTATAAATTTCTTCTCAAA ACTAGCTGGTGTGCTTCTAACGAAGCACTCAAGAAGAATG AGGGAATTGTCAATCAGTTTATAACCATCAGGAATGATCA AAGGCAGTCCCGGGCACACAATCCCAGACTCTATTAGAAT TGCCTCAACAGATTTATCATCATGGTTGTGTATGCAGCCG CTCTTGTCAGCACTGTCTATCTCTATACAACGCGACAAAA GTTTGAGTCCCTCTATCAATACCATTCTGGGTTCTCTTTG CCCTAAAAAGTTGAGCTTCTGCCTTGACAACCTCTCATCT TGTTCTATGTGGTTTAAGCACAACTCTCTCAACTCCGAAA TAGCCTCATCCATTGCGCATCAAAAAGCCTAGGATCCTCG GTGCG
14	lymphocytic choriomeningitis strain MP segment S, complete sequence (The genomic segment is RNA, the sequence in SEQ ID NO:14 is shown for DNA; however, exchanging all thymidines ("T") in SEQ ID NO:14 for uridines ("U") provides the RNA sequence.)	CGCACCGGGGATCCTAGGCTTTTTGGATTGCGCTTTCCTC AGCTCCGTCTTGTGGGAGAATGGGTCAAATTGTGACGATG TTTGAGGCTCTGCCTCACATCATTGATGAGGTCATTAACA TTGTCATTATCGTGCTTATTATCATCACGAGCATCAAAGC TGTGTACAATTTCGCCACCTGCGGGATACTTGCATTGATC AGCTTTCTTTTTCTGGCTGGCAGGTCCTGTGGAATGTATG GTCTTGATGGGCCTGACATTTACAAAGGGGTTTACCGATT CAAGTCAGTGGAGTTTGACATGTCTTACCTTAACCTGACG ATGCCCAATGCATGTTCGGCAAACAACTCCCATCATTATA TAAGTATGGGGACTTCTGGATTGGAGTTAACCTTCACAAA TGACTCCATCATCACCCACAACTTTTGTAATCTGACTTCC GCCCTCAACAAGAGGACTTTTGACCACACACTTATGAGTA TAGTCTCAAGTCTGCACCTCAGCATTAGAGGGGTCCCCAG

SEQ ID NO:	Description	Sequence
		CTACAAAGCAGTGTCCTGTGATTTTAACAATGGCATCACT ATTCAATACAACCTGTCATTTTCTAATGCACAGAGCGCTC TGAGTCAATGTAAGACCTTCAGGGGAGAGTCCTGGATAT GTTCAGAACTGCTTTTGGAGGAAAGTACATGAGGAGTGGC TGGGGCTGGACAGGTTCAGATGGCAAGACTACTTGGTGCA GCCAGACAAACTACCAATATCTGATTATACAAAACAGGAC TTGGGAAAACCACTGCAGGTACGCAGGCCCTTTCGGAATG TCTAGAATTCTCTTCGCTCAAGAAAAGACAAGGTTTCTAA CTAGAAGGCTTGCAGGCACATTCACTTGGACTTTATCAGA CTCATCAGGAGTGGAGAATCCAGGTGGTTACTGCTTGACC AAGTGGATGATCCTCGCTGCAGAGCTCAAGTGTTTTGGGA ACACAGCTGTTGCAAAGTGCAATGTAAATCATGATGAAGA GTTCTGTGATATGCTACGACTGATTGATTACAACAAGGCT GCTTTGAGTAAATTCAAAGAAGATGTAGAATCCGCTCTAC ATCTGTTCAAGACAACAGTGAATTCTTTGATTTCTGATCA GCTTTTGATGAGAAATCACCTAAGAGACTTGATGGGAGTG CCATACTGCAATTACTCGAAATTCTGGTATCTAGAGCATG CAAAGACTGGTGAGACTAGTGTCCCCAAGTGCTGGCTTGT CAGCAATGGTTCTTATTTGAATGAAACCCATTTCAGCGAC CAAATTGAGCAGGAAGCAGATAATATGATCACAGAAATGC TGAGAAAGGACTACATAAAAAGGCAAGGGAGTACCCCTCT AGCCTTGATGGATCTATTGATGTTTTCTACATCAGCATAT TTGATCAGCATCTTTCTGCATCTTGTGAGGATACCAACAC ACAGACACATAAAGGGCGGCTCATGCCCAAAACCACATCG GTTAACCAGCAAGGGAATCTGTAGTTGTGGTGCATTTAAA GTACCAGGTGTGGAAACCACCTGGAAAAGACGCTGAACAG CAGCGCCTCCCTGACTCACCACCTCGAAAGAGGTGGTGAG TCAGGGAGGCCCAGAGGGTCTTAGAGTGTTACGACATTTG GACCTCTGAAGATTAGGTCATGTGGTAGGATATTGTGGAC AGTTTTCAGGTCGGGGAGCCTTGCCTTGGAGGCGCTTTCA AAGATGATACAGTCCATGAGTGCACAGTGTGGGGTGACCT

SEQ ID NO:	Description	Sequence
		CTTTCTTTTTCTTGTCCCTCACTATTCCAGTGTGCATCTT GCATAGCCAGCCATATTTGTCCCAGACTTTGTCCTCATAT TCTCTTGAAGCTTCTTTAGTCATCTCAACATCGATGAGCT TAATGTCTCTTCTGTTTTGTGAATCTAGGAGTTTCCTGAT GTCATCAGATCCCTGACAACTTAGGACCATTCCCTGTGGA AGAGCACCTATTACTGAAGATGTCAGCCCAGGTTGTGCAT TGAAGAGGTCAGCAAGGTCCATGCCATGTGAGTATTTGGA GTCCTGCTTGAATTGTTTTTGATCAGTGGGTTCTCTATAG AAATGTATGTACTGCCCATTCTGTGGCTGAAATATTGCTA TTTCTACCGGGTCATTAAATCTGCCCTCAATGTCAATCCA TGTAGGAGCGTTAGGGTCAATACCTCCCATGAGGTCCTTC AGCAACATTGTTTGGCTGTAGCTTAAGCCCACCTGAGGTG GGCCCGCTGCCCCAGGCGCTGGTTTGGGTGAGTTGGCCAT AGGCCTCTCATTTGTCAGATCAATTGTTGTGTTCTCCCAT GCTCTCCCTACAACTGATGTTCTACAAGCTATGTATGGCC ACCCCTCCCCTGAAAGACAGACTTTGTAGAGGATGTTCTC GTAAGGATTCCTGTCTCCAACCTGATCAGAAACAAACATG TTGAGTTTCTTCTTGGCCCCAAGAACTGCTTTCAGGAGAT CCTCACTGTTGCTTGGCTTAATTAAGATGGATTCCAACAT GTTACCCCCATCTAACAAGGCTGCCCCTGCTTTCACAGCA GCACCGAGACTGAAATTGTAGCCAGATATGTTGATGCTAG ACTGCTGCTCAGTGATGACTCCCAAGACTGGGTGCTTGTC TTTCAGCCTTTCAAGGTCACTTAGGTTCGGGTACTTGACT GTGTAAAGCAGCCCAAGGTCTGTGAGTGCTTGCACAACGT CATTGAGTGAGGTTTGTGATTGTTTGGCCATACAAGCCAT TGTTAAGCTTGGCATTGTGCCGAATTGATTGTTCAGAAGT GATGAGTCCTTCACATCCCAGACCCTCACCACACCATTTG CACTCTGCTGAGGTCTCCTCATTCCAACCATTTGCAGAAT CTGAGATCTTTGGTCAAGCTGTTGTGCTGTTAAGTTCCCC ATGTAGACTCCAGAAGTTAGAGGCCTTTCAGACCTCATGA TTTTAGCCTTCAGTTTTTCAAGGTCAGCTGCAAGGGACAT

SEQ ID NO:	Description	Sequence
		CAGTTCTTCTGCACTAAGCCTCCCTACTTTTAGAACATTC TTTTTTGATGTTGACTTTAGGTCCACAAGGGAATACACAG TTTGGTTGAGGCTTCTGAGTCTCTGTAAAATCTTTGTCATC CCTCTTCTCTTTCCTCATGATCCTCTGAACATTGCTCACC TCAGAGAAGTCTAATCCATTCAGAAGGCTGGTGGCATCCT TGATCACAGCAGCTTTCACATCTGATGTGAAGCCTTGAAG CTCTCTCCTCAATGCCTGGGTCCATTGAAAGCTTTTAACT TCTTTGGACAGAGACATTTTGTCACTCAGTGGATTTCCAA GTCAAATGCGCAATCAAAATGCCTAGGATCCACTGTGCG
15	amino acid sequence of the NP protein of the MP strain of LCMV	MSLSKEVKSFQWTQALREELQGFTSDVKAAVIKDATSLLN GLDFSEVSNVQRIMRREKRDDKDLQRLRSLNQTVYSLVDL KSTSKKNVLKVGNLSAEELMSLAADLEKLKAKIMRSERPL TSGVYMGNLTAQQLDQRSQILQMVGMRRPCQSANGVVKVW DVKDSSLLNNQFGTMPSLTMACMAKQSQTSLNDVVQALTD LGLLYTVKYPNLSDLERLKDKHPVLGVITEQQSSINISGY NFSLGAAVKAGAALLDGGNMLESILIKPSNSEDLLKAVLG AKKKLNMFVSDQVGDRNPYENILYKVCLSGEGWPYIACRT SVVGRAWENTTIDLTNEPPMANSPKPAPGAAGPFQVGLSY SQTMLLKDLMGGIDPNAPTWIDIEGRFNDPVEIAIFQPQN GQYIHFYREPTDQKQFKQDSKYSHGMDLADLFNAQPGLTS SVIGALPQGMVLSCQGSDDIRKLLDSQNERDIKLIDVEMT KEASREYEDKVWDKYGWLCKMHTGIVRDKKKEVTPHCAL MDCIIFESASKARLPDLKTVHNILPHDLIFRGPNVVTL
16	amino acid sequence of the GP protein of the MP strain of LCMV	MGQIVTMFEALPHIIDEVINIVIIVLIIITSIKAVYNFAT CGILALISFLFLAGRSCGMYGLDGPDIYKGVYRFKSVEFD MSYLNLTMPNACSANNSHHYISMGTSGLELTFTNDSIITH NFCNLTSALNKRTFDHTLMSIVSSLHLSIRGVPSYKAVSC DFNNGITIQYNLSFSNAQSALSQCKTFRGRVLDMFRTAFG GKYMKSGWGWTGSDGKTTWCSQTNYQYLIIQNRTWENHCR YAGPFGMSRILFAQEKTRFLTRRLAGTFTWTLSDSSGVEN PGGYCLTKWMILAAELKCFGNTAVAKCNVNHDEEFCDMLR
		LIDYNKAALSKFKEDVESALHLFKTTVNSLISDQLLMRNH LRDLMGVPYCNYSKFWYLEHAKTGETSVPKCWLVSNGSYL NETHFSDQIEQEADNMITEMLRKDYIKRQGSTPLALMDLL MFSTSAYLISIFLHLVRIPTHRHIKGGSCPKPHRLTSKGI CSCGAFKVPGVETTWKRR

SEQ ID NO:	Description	Sequence
17	amino acid sequence of the L protein of the MP strain of LCMV	MDEAISELRELCLNHIEQDERLSRQKLNFLGQREPRMVLI EGLKLLSPCIEIDSADKSGCIHNHDDKSVEAILIESGIVC PGLPLIIPDGYKLIDNSLILLECFVRSTPASPEKKFIEDT NKLACIKEDLAIAGITLVPIVDGRCDYDNSFMPEWVNFKF RDLLFKLLEYSSQDEKVFEESEYFRLCESLKTTVDKRSGI DSMEILKDARSFHNDEIMEMCHDGVNPNMNCDDVVLGINS LYSRFRRDLETGKLKRSFQKINPGNLIKEFSELYETLADS DDISALSKEAVESCPLMRFITADTHGYERGSETSTEYERL LSMLNKVKSLKLLNTRREQLLNLDVLCLSSLIKQSKLKGS KNDKEWVGCCYGSVNDRLVSFHSTKEEFIRLLRNERKSKA YRKVSLEDLFRTSINEFILKVQECLSVVGLSFGHYGLSEH LEHECHIPFIRFENFMRSGTHPIMYYTKFEDYDFQPNTEQ LRNMHSLKRLSSVCLALTNSMKTSSVARLRQNQLGSVRYQ VVECKEVFCQVIKLDSEEYHLLYQKTGESSRCYSIQGPNG ELISFYADPKRFFLPIFSDEVLHNMIDTMISWIRSCPDLK DSIDDVEIALRTLLLLMLTNPTKRNQKQVQNIRYLVMAIV SDFSSTSLMDKLKEDLITPAEKVVYKLLRFLIKTVFGTGE KVLLSAKFKFMLNVSYLCHLITKETPDRLTDQIRCFERFF EPKSEFGFFVNPKESITPEEECVFYDQMKKFTGKEVDCQR TTPGVNLEAFSMMVSSFNNGTLIFKGEKPLNSLDPMTNSG CATALDLASNKSVVVNKHLNGERLLEYDFNKLLVSAVSQI TESFMRKQKYKLNHSDYEYKVSKLVSRLVIGSKETEAGKL EGDSADICFDGEEETSFFKNLEDKVNSTIKRYERSKKTNE GENEVGFENTKGLHELQTILSGKMAYLRKVILSEISFHLV EDFDPSCLTNDDMKFICEAIETSTELSPLYFTSAVKEQCG LDEMAKNLCRKFFSEGDWFSCMKMILQMNANAYSGKYRH

SEQ ID NO:	Description	Sequence
		MQRQGLNFKFDWDKLEEDVRISERESNSESLSKALSLTKC MSAALKNLCFYSEESPTSYTSVGPDSGRLKFALSYKEQVG GNRELYIGDLRTKMFTRLIEDYFESFSSFFSGSCLNNDKE FENAILSMTINVREGLLNYSMDHSKWGPMMCPFLFLMLLQ NLKLGDDQIVRSGKDHISTLLTWHMHKLVEVPFPVVNAMM KSYIKSKLKLLRGSETTVTERIFREYFELGIVPSHISSLI DMGQGILHNASDFYGLISERFINYCIGVIFGERPESYTSS DDQITLFDRRLSELVDSDFEEVLVLLEFHSHLSGLLNKFI SPKSVVGRFAAEFKSRFYVWGEEVPLLTKFVSAALHNVKC KEPHQLCETIDTIADQAVANGVPVSLVNCIQKRTLDLLKY ANFPLDPFLLNTNTDVKIDWLDGSRGYRIQRLIEELCPSET KVMRRLVRRLHHKLKNGEFNEEFFLDLFNRDKKEAILQLG NILGLEEDLSQLANINWLNLNELFPLPMVLPQKVVYPSVM TFQEERIPSLIKTLQNKLCSKFTRGAQKLLSEAINKSAFQ SCISSGFIGLCKTLGSRCVRNKNRDNLYIRKVLEDLAMDA HVTAIHRHDGIMLYICDRQSHPEANCDHISLLRPLLWDYI CISLSNSFELGVWVLAEFVKGKNEGSSSLKHLNFCDYVAR KPESSRLLEDKISLNHVIQSVRRLYPKIYEDQLLPFMSDM SSKNMRWSPRIKFLDLCVLIDINSESLSLISHVVKWKRDE HYTVLFSDLVNSHQRSDSSLVDEFVVSERDVCKNFLKQVY FESFVREFVATSPTLGSFSWFPHKDMMPSEDGAEALGPFQ SFILKVVNKNMERPMFRNDLQFGFGWFSYRLGDIVCNAAM LIKQGLTNPKAFKSLRNLWDYMINNTEGVLEFSITVDFTH NQNNTDCLRKFSLIFLVKCQLQGPGVAEFLSCSHLFKGEV DRRFLDECLSLLRSDSIFKVNDGVFDIRSEEFEDYMEDFL ILGDSLELELIGSRKILDGIRSLDFERIGPEWEPVFLTVR MGALFEGRSLVQNIVVKLETKDMRVFLAELEGYGNFDDVL GSLLLHSFRTGEHLQGSEISTILQELCIDRSILLVPLSLV PDWFTPKDCRLCFSKSKNTVMYETVVGKYPLKGKSCDDWL TKSVVEEID
18	amino acid sequence of	MGQGKSKEGRDASNTSRAEILPDTTYLGPLNCKSCWQRFD
	the Z protein of the MP strain of LCMV	SLVRCHDSYLCRHCLNLLLSVSDRCPLCKHPLPTKLKIST APSSPPPYEE

SEQ ID NO:	Description	Sequence
19	Junin virus Candid# 1 L segment	GCGCACCGGGGATCCTAGGCGTAACTTCATCATTAAAATCT CAGATTCTGCTCTGAGTGTGACTTACTGCGAAGAGGCAGAC AAATGGGCAACTGCAACGGGGCATCCAAGTCTAACCAGCCA GACTCCTCAAGAGCCACACAGCCAGCCGCAGAATTTAGGAG GGTAGCTCACAGCAGTCTATATGGTAGATATAACTGTAAGT GCTGCTGGTTTGCTGATACCAATTTGATAACCTGTAATGAT CACTACCTTTGTTTAAGGTGCCATCAGGGTATGTTAAGGAA TTCAGATCTCTGCAATATCTGCTGGAAGCCCCT GCCCACCACAATCACAGTACCGGTGGAGCCAACAGCACCAC CACCATAGGCAGACTGCACAGGGTCAGACCCGACCCCCCGG GGGGCCCCCATGGGGACCCCCCGTGGGGGAACCCCGGGGGT GATGCGCCATTAGTCAATGTCTTTGATCTCGACTTTGTGCT TCAGTGGCCTGCATGTCACCCCTTTCAATCTGAACTGCCCT TGGGGATCTGATATCAGCAGGTCATTTAAAGATCT GCTGAATGCCACCTTGAAATTTGAGAATTCCAACCAGTCAC CAAATTTATCAAGTGAACGGATCAACTGCTCTTTGTGTA GATCATAAACGAGGACAAAGTCCTCTTGCTGAAATAATATT GTTTGTGATGTTGTTTTTAGATAAGGCCATAGTTGGCTT AATAAGGTTTCCACACTATCAATGTCCTCTAGTGCTCCAAT TGCCTTGACTATGACATCCCCAGACAACTCAACTCTATA TGTTGACAACCTTTCATTACCTCTGTAAAAGATACCCTCTT TCAAGACAAGAGGTTCTCCTGGGTTATCTGGCCCAATGA GGTCATATGCATACTTGTTACTTAGTTCAGAATAAAAGTCA CCAAAGTTGAACTTAACATGGCTCAGAATATTGTCATCA TTTGTCGCAGCGTAGCCTGCATCAATAAACAAGCCAGCTAG GTCAAAGCTCTCATGGCCTGTGAACAATGGTAGGCTAGC GATAACCAGTGCACCATCCAACAATGAGTGGCTTCCCTCAG ACCCAGAAACACATTGACTCATTGCATCCACATTCAGCT CTAATTCAGGGGTACCGACATCATCCACTCCTAGTGAACTG

SEQ ID NO:	Description	Sequence
		ACAATGGTGTAACTGTACACCATCTTTCTTCTAAGTTTA AATTTTGTCGAAACTCGTGTGTGTTCTACTTGAATGATCAA TTTTAGTTTCACAGCTTCTTGGCAAGCAACATTGCGCAA CACAGTGTGCAGGTCCATCATGTCTTCCTGAGGCAACAAGG AGATGTTGTCAACAGAGACACCCTCAAGGAAAACCTTGA TATTATCAAAGCTAGAAACTACATAACCCATTGCAATGTCT TCAACAAACATTGCTCTTGATACTTTATTATTCCTAACT GACAAGGTAAAATCTGTGAGTTCAGCTAGATCTACTTGACT GTCATCTTCTAGATCTAGAACTTCATTGAACCAAAAGAA GGATTTGAGACACGATGTTGACATGACTAGTGGGTTTATCA TCGAAGATAAGACAACTTGCACCATGAAGTTCCTGCAAA CTTGCTGTGGGCTGATGCCAACTTCCCAATTTGTATACTCT GACTGTCTAACATGGGCTGAAGCGCAATCACTCTGTTTC ACAATATAAACATTATTATCTCTTACTTTCAATAAGTGACT TATAATCCCTAAGTTTTCATTCATCATGTCTAGAGCCAC ACAGACATCTAGAAACTTGAGTCTTCCACTATCCAAAGATC TGTTCACTTGAAGATCATTCATAAAGGGTGCCAAATGTT CTTCAAATAGTTTGGGGTAATTTCTTCGTATAGAATGCAAT ACATGGTTCATGCCTAATTGGTCTTCTATCTGTCGTACT GCTTTGGGTTTAACAGCCCAGAAGAAATTCTTATTACATAA GACCAGAGGGGCCTGTGGACTCTTAATAGCAGAAAACAC CCACTCCCCTAACTCACAGGCATTTGTCAGCACCAAAGAGA AGTAATCCCACAAAATTGGTTTAGAAAATTGGTTAACTT CTTTAAGTGATTTTTGACAGTAAATAACTTTAGGCTTTCTC TCACAAATTCCACAAAGACATGGCATTATTCGAGTAAAT ATGTCCTTTATATACAGAAATCCGCCTTTACCATCCCTAAC ACACTTACTCCCCATACTCTTACAAAACCCAATGAAGCC TGAGGCAACAGAAGACTGAAATGCAGATTTGTTGATTGACT CTGCCAAGATCTTCTTCACGCCTTTTGTGAAATTTCTTG ACAGCCTGGACTGTATTGTCCTTATCAATGTTGGCATCTCT TCTTTCTCTAACACTCTTCGACTTGTCATGAGTTTGGTC

SEQ ID NO:	Description	Sequence
		CTCAAGACCAACCTCAAGTCCCCAAAGCTCGCTAAATTGAC CCATCTGTAGTCTAGAGTTTGTCTGATTTCATCTTCACT ACACCCGGCATATTGCAGGAATCCGGATAAAGCCTCATCCC CTCCCCTGCTTATCAAGTTGATAAGGTTTTCCTCAAAGA TTTTGCCTCTCTTAATGTCATTGAACACTTTCCTCGCGCAG TTCCTTATAAACATTGTCTCCTTATCATCAGAAAAAATA GCTTCAATTTTCCTCTGTAGACGGTACCCTCTAGACCCATC AACCCAGTCTTTGACATCTTGTTCTTCAATAGCTCCAAA CGGAGTCTCTCTGTATCCAGAGTATCTAATCAATTGGTTGA CTCTAATGGAAATCTTTGACACTATATGAGTGCTAACCC CATTAGCAATACATTGATCACAAATTGTGTCTATGGTCTCT GACAGTTGTGTTGGAGTTTTACACTTAACGTTGTGTAGA GCAGCAGACACAAACTTGGTGAGTAAAGGAGTCTCTTCACC CATGACAAAAAATCTTGACTTAAACTCAGCAACAAAAGTTC CTATCACACTCTTTGGGCTGATAAACTTGTTTAATTTAGAA GATAAGAATTCATGGAAGCACACCATTTCCAGCAGTT CTGTCCTGTCTTGAAACTTTTCATCACTAAGGCAAGGAATT TTTATAAGGCTAACCTGGTCATCGCTGGAGGTATAAGTG ACAGGTATCACATCATACAATAAGTCAAGTGCATAACACAG AAATTGTTCAGTAATTAGCCCATATAAATCTGATGTGTT GTGCAAGATTCCCTGGCCCATGTCCAAGACAGACATTATAT GGCTGGGGACCTGGTCCCTTGACTGCAGATACTGGTGAA AAAACTCTTCACCAACACTAGTACAGTCACAACCCATTAAA CCTAAAGATCTCTTCAATTTCCCTACACAGTAGGCTTCT GCAACATTAATTGGAACTTCAACGACCTTATGAAGATGCCA TTTGAGAATGTTCATTACTGGTTCAAGATTCACCTTTGT TCTATCTCTGGGATTCTTCAATTCTAATGTGTACAAAAAAG AAAGGAAAAGTGCTGGGCTCATAGTTGGTCCCCATTTGG AGTGGTCATATGAACAGGACAAGTCACCATTGTTAACAGCC ATTTTCATATCACAGATTGCACGTTCGAATTCCTTTTCT GAATTCAAGCATGTGTATTTCATTGAACTACCCACAGCTTC

SEQ ID NO:	Description	Sequence
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SEQ ID NO:	Description	Sequence
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SEQ ID NO:	Description	Sequence
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20	Junin virus Candid# 1 S segment	GCGCACCGGGGATCCTAGGCGATTTTGGTTACGCTATAATT GTAACTGTTTTCTGTTTGGACAACATCAAAAACATCCATTG CACAATGGGGCAGTTCATTAGCTTCATGCAAGAAATACCAA CCTTTTTGCAGGAGGCTCTGAACATTGCTCTTGTTGC AGTCAGTCTCATTGCCATCATTAAGGGTATAGTGAACTTGT ACAAAAGTGGTTTATTCCAATTCTTTGTATTCCTAGCGC

SEQ ID NO:	Description	Sequence
		TTGCAGGAAGATCCTGCACAGAAGAAGCTTTCAAAATCGGA
		CTGCACACTGAGTTCCAGACTGTGTCCTTCTCAATGGTG
		GGTCTCTTTTCCAACAATCCACATGACCTACCTTTGTTGTG
		TACCTTAAACAAGAGCCATCTTTACATTAAGGGGGGCAA
		TGCTTCATTTCAGATCAGCTTTGATGATATTGCAGTATTGT
		TGCCACAGTATGATGTTATAATACAACATCCAGCAGATA
		TGAGCTGGTGTTCCAAAAGTGATGATCAAATTTGGTTGTCT
		CAGTGGTTCATGAATGCTGTGGGACATGATTGGCATCTA
		GACCCACCATTTCTGTGTAGGAACCGTGCAAAGACAGAAGG
		CTTCATCTTTCAAGTCAACACCTCCAAGACTGGTGTCAA
		TGGAAATTATGCTAAGAAGTTTAAGACTGGCATGCATCATT
		TATATAGAGAATATCCTGACCCTTGCTTGAATGGCAAAC
		TGTGCTTAATGAAGGCACAACCTACCAGTTGGCCTCTCCAA
		TGTCCACTCGACCACGTTAACACATTACACTTCCTTACA
		AGAGGTAAAAACATTCAACTTCCAAGGAGGTCCTTGAAAGC
		ATTCTTCTCCTGGTCTTTGACAGACTCATCCGGCAAGGA
		TACCCCTGGAGGCTATTGTCTAGAAGAGTGGATGCTCGTAG
		CAGCCAAAATGAAGTGTTTTGGCAATACTGCTGTAGCAA
		AATGCAATTTGAATCATGACTCTGAATTCTGTGACATGTTG
		AGGCTCTTTGATTACAACAAAAATGCTATCAAAACCCTA
		AATGATGAAACTAAGAAACAAGTAAATCTGATGGGGCAGAC
		AATCAATGCCCTGATATCTGACAATTTATTGATGAAAAA
		CAAAATTAGGGAACTGATGAGTGTCCCTTACTGCAATTACA
		CAAAATTTTGGTATGTCAACCACACACTTTCAGGACAAC
		ACTCATTACCAAGGTGCTGGTTAATAAAAAACAACAGCTAT
		TTGAACATCTCTGACTTCCGTAATGACTGGATATTAGAA
		AGTGACTTCTTAATTTCTGAAATGCTAAGCAAAGAGTATTC
		GGACAGGCAGGGTAAAACTCCTTTGACTTTAGTTGACAT
		CTGTATTTGGAGCACAGTATTCTTCACAGCGTCACTCTTCC
		TTCACTTGGTGGGTATACCCTCCCACAGACACATCAGGG
		GCGAAGCATGCCCTTTGCCACACAGGTTGAACAGCTTGGGT

SEQ ID NO:	Description	Sequence
		GGTTGCAGATGTGGTAAGTACCCCAATCTAAAGAAACCA ACAGTTTGGCGTAGAGGACACTAAGACCTCCTGAGGGTCCC CACCAGCCCGGGCACTGCCCGGGCTGGTGTGGCCCCCCAGT CCGCGGCCTGGCCGCGGACTGGGGAGGCACTGCTTACAGTG CATAGGCTGCCTTCGGGAGGAACAGCAAGCTCGGTGGTAAT AGAGGTGTAGGTTCCTCCTCATAGAGCTTCCCATCTAGCAC TGACTGAAACATTATGCAGTCTAGCAGAGCACAGTGTGGTT CACTGGAGGCCAACTTGAAGGGAGTATCCTTTTCCCTCTTT TTCTTATTGACAACCACTCCATTGTGATATTTG CATAAGTGACCATATTTCTCCCAGACCTGTTGATCAAACTG CCTGGCTTGTTCAGATGTGAGCTTAACATCAACCAGTTT AAGATCTCTTCTTCCATGGAGGTCAAACAACTTCCTGATGT CATCGGATCCTTGAGTAGTCACAACCATGTCTGGAGGCA GCAAGCCGATCACGTAACTAAGAACTCCTGGCATTGCATCT TCTATGTCCTTCATTAAGATGCCGTGAGAGTGTCTGCTA CCATTTTTAAACCCTTTCTCATCATGTGGTTTTCTGAAGCA GTGAATGTACTGCTTACCTGCAGGTTGGAATAATGCCAT CTCAACAGGGTCAGTGGCTGGTCCTTCAATGTCGAGCCAAA GGGTGTTGGTGGGGTCGAGTTTCCCCACTGCCTCTCTGA TGACAGCTTCTTGTATCTCTGTCAAGTTAGCCAATCTCAAA TTCTGACCGTTTTTTTCCGGCTGTCTAGGACCAGCAACT GGTTTCCTTGTCAGATCAATACTTGTGTTGTCCCATGACCT GCCTGTGATTTGTGATCTAGAACCAATATAAGGCCAACC ATCGCCAGAAAGACAAAGTTTGTACAAAAGGTTTTCATAAG GATTTCTATTGCCTGGTTTCTCATCAATAAACATGCCTT CTCTTCGTTTAACCTGAATGGTTGATTTTATGAGGGAAGAG AAGTTTTCTGGGGTGACTCTGATTGTTTCCAACATGTTT CCACCATCAAGAATAGATGCTCCAGCCTTTACTGCAGCTGA AAGACTGAAGTTGTAACCAGAAATATTGATGGAGCTTTC ATCTTTAGTCACAATCTGAAGGCAGTCATGTTCCTGAGTCA GTCTGTCAAGGTCACTTAAGTTTGGATACTTCACAGTGT

SEQ ID NO:	Description	Sequence
		ATAGAAGCCCAAGTGAGGTTAAAGCTTGTATGACACTGTTC ATTGTCTCACCTCCTTGAACAGTCATGCATGCAATTGTC AATGCAGGAACAGAGCCAAACTGATTGTTTAGCTTTGAAGG GTCTTTAACATCCCATATCCTCACCACACCATTTCCCCC AGTCCCTTGCTGTTGAAATCCCAGTGTTCTCAATATCTCTG ATCTTTTAGCAAGTTGTGACTGGGACAAGTTACCCATGT AAACCCCCTGAGAGCCTGTCTCTGCTCTTCTTATCTTGTTT TTTAATTTCTCAAGGTCAGACGCCAACTCCATCAGTTCA TCCCTCCCCAGATCTCCCACCTTGAAAACTGTGTTTCGTTG AACACTCCTCATGGACATGAGTCTGTCAACCTCTTTATT CAGGTCCCTCAACTTGTTGAGGTCTTCTTCCCCCTTTTTAG TCTTTCTGAGTGCCCGCTGCACCTGTGCCACTTGGTTGA AGTCGATGCTGTCAGCAATTAGCTTGGCGTCCTTCAAAACA TCTGACTTGACAGTCTGAGTGAATTGGCTCAAACCTCTC CTTAAGGACTGAGTCCATCTAAAGCTTGGAACCTCCTTGGA GTGTGCCATGCCAGAAGTTCTGGTGATTTTGATCTAGAA TAGAGTTGCTCAGTGAAAGTGTTAGACACTATGCCTAGGAT CCACTGTGCG
21	amino acid sequence of the NP protein of the Clone 13 strain of LCMV (GenBank Accession No. ABC96002.1; GI:86440166)	MSLSKEVKSFQWTQALRRELQSFTSDVKAAVIKDATNLLNG LDFSEVSNVQRIMRKEKRDDKDLQRLRSLNQTVHSLVDLKS TSKKNVLKVGRLSAEELMSLAADLEKLKAKIMRSERFQASG VYMGNLTTQQLDQRSQILQIVGMRKPQQGASGVVRVWDVKD SSLLNNQFGTMPSLTMACMAKQSQTPLNDVVQALTDLGLLY TVKYPNLNDLERLKDKHPVLGVITEQQSSINISGYNFSLGA AVKAGAALLDGGNMLESILIKPSNSRDLLKAVLGAKRKLNM FVSDQVGDRNPYENILYKVCLSGEGWPYIACRTSIVGRAWE NTTIDLTSEKPAVNSPRPAPGAAGPPQVGLSYSQTMLLKDL MGGIDPNAPTWIDIEGRFNDPVEIAIFQPQNGQFIHFYREP VDQKQFKQDSKYSHGMDLADLFNAQPGLTSSVIGALPQGMV LSCQGSDDIRKLLDSQNRKDIKLIDVEMTREASREYEDKVW DKYGWLCKMHTGIVRDKKKKEITPHCALMDCIIFESASKAR
		LPDLKTVHNILPHDLIFRGPNVVTL

SEQ ID NO:	Description	Sequence
22	amino acid sequence of the GP protein of the Clone 13 strain of LCMV (GenBank Accession No. ABC96001.2; GI:116563462)	MGQIVTMFEALPHIIDEVINIVIIVLIVITGIKAVYNFATC GIFALISFLLLAGRSCGMYGLKGPDIYKGVYQFKSVEFDMS HLNLTMPNACSANNSHEYISMGTSGLELTFTNDSIISHNFC NLTSAFNKKTFDHTLMSIVSSLHLSIRGNSNYKAVSCDFNN GITIQYNLTFSDAQSAQSQCRTFKGRVLDMFRTAFGGKYMR SGWGWTGSDGKTTWCSQTSYQYLIIQNRTWENHCTYAGPFG MSRILLSQEKTKFLTRRLAGTFTWTLSDSSGVENPGGYCLT KWMILAAELKCFGNTAVAKCNVNHDEEFCDMLRLIDYNKAA LSKFKEDVESALHLFKTTVNSLISDQLLMRNHLRDLMGVPY CNYSKFWYLEHAKTGETSVPKCWLVTNGSYLNETHFSDQIE QEADNMITEMLRKDYIKRQGSTPLALMDLLMFSTSAYLVSI FLHLVKIPTHPHIKGGSCPKPHRLTNKGICSCGAFKVPGVK TVWKRR
23	amino acid sequence of the L protein of the Clone 13 strain of LCMV (GenBank Accession No. ABC96004.1; GI:86440169)	MDEIISELPELCLNYIEQDERLSPQKLNFLGQREPRMVLIE GLKLLSRCIEIDSADKSGCTHNHDDKSVETILVESGIVCPG LPLIIPDGYKLIDNSLILLECFVRSTPASFEKKFIEDTNKL ACIREDLAVAGVTLVPIVDGRCDYDNSFMPEWANFKFRDLL FKLLEYSNQNEKVFEESEYFRLCESLKTTIDKRSGMDSMKI LKDARSTHNDEIMRMCREGINPNMSCDDVVFGINSLPSRFR RDLESGKLKRNFQKVNPEGLIKEFSELYENLADSDDILTLS REAVESCPLMRFITAETHGHERGSETSTEYERLLSMLNKVK SLKLLNTRREQLLNLDVLCLSSLIKQSKFRGLKNDKHWVGC CYSSVNDRLVSFSSTKEEFIRLLRNPKKSKVFRKVSFEELF RASISEFIAKIQKCLLVVGLSFEHYGLSEHLEQECHIPFTE FENFMKIGAHPIMYYTKFEDYNFQFSTEQLKNIQSLPRLSS VCLALTNSMKTSSVARLRQNQIGSVRYQVVECKEVFCQVIK LDSEEYHLLYQKTGESSRCYSIQGPDGRLISFYADPKRFFL PIFSDEVLYNMIDIMISWIRSCFDLKDCLTDIEVALRTLLL LMLTNPTKRNQKQVQSVRYLVMAIVSDFSSTSLMDKLREDL ITPAEKVVYKLLEFLIKTIFGTGEKVLLSAKFKFMLNVSYL

SEQ ID NO:	Description	Sequence
		CHLITKETPDRLTDQIKCFEKFFEPKSQFGFFVNPKEAITP EEECVFYEQMKRFTSKEIDCQATTPGVNLEAFSLMVSSFNN GTLIFKGEKKLNSLDPMTNSGCATALDLASNKSVVVNKHLN GERLLEYDFNKLLVSAVSQITESFVRKQKYKLSHSDYEYKV SKLVSRLVIGSKGEETGRSEDNLAEICFDGEEETSFFKSLE EKVNTTIAPYRRGERANDKGLGEKLTNTKGLHHLQLILTGK MAHLRKVILSEISFHLVEDFDPSCLTNDDMKFICEAVEGST ELSPLYFTSVIKDQCGLDEMAKNLCSKFFSENDWFSCMKMI LLQMNANAYSGKYRHMQRQGLNFKFLWDKLEEDVRISERES NSESLSKALSLTQCMSAALKNLCFYSEESPTSYTSVGPDSG RLKFALSYKEQVGGNRELYIGDLRTKMFTRLIEDYFESFSS FFSGSCLNNDKEFENAILSMTINVREGFLNYSMDHSKWGPM MCPFLFLMFLQNLKLGDDQYVRSGKDHVSTLLTWHMSKLVE VPFPVVNAMMKSYVKSKLKLLRGSETTVTERIFRQYFEMGI VPSHISSLIDMSQGILHNASDFYGLLSEFFINYCIGVIFGE RPEAYTSSDDQITLFDRRLSDLVVSDPEEVLVLLEFQSHLS GLLNKFISPKSVAGRFAAEFKSRFYVWGEEVPLLTKFVSAA LHNVKCKEPHQLCETIDTIADQAIANGVPVSLVNSIQRRTL DLLKYANFFLDPFLLNENTDVKDWLDGSRGYRIQRLIEELC PNETKVVRKLVRKLHHKLKNGEFNEEFFLDLFNRDKKEAIL QLGDLLGLEEDLNQLADVNWLNLNEMFPLRMVLRQKVVYPS VMTFQEERIPSLIKTLQNKLCSKFTRGAQKLLSEAINKSAF QSCISSGFIGLCKTLGSRCVRNKNRENLYIKKLLEDLTTDD HVTRVCNRDGITLYICDKQSHPEAHRDHICLLRPLLWDYIC ISLSNSFELGVWVLAEPTKGKNNSENLTLKHLNPCDYVARK PESSRLLEDKVNLNQVIQSVRRLYPKIFEDQLLPFMSDMSS KNMSWSPRIKFLDLCVLIDINSESLSLISHVVKWKRDEHYT VLFSDLANSHQRSDSSLVDEFVVSTRDVCKNFLKQVYFESF VREFVATTRTLGNFSWFPHKEMMPSEDGAEALGPFQSFVSK VVNKNVERFMFRNDLQFGFGWFSYRMGDVVCNAAMLIRQGL TNPKAFKSLKDLWDYMLNYTKGVLEFSISVDFTHNQNNTDC
		LRKFSLIFLVPCQLQNPGVAELLSCSHLFKGEIDRRMLDEC LHLLRTDSVFKVNDGVFDIRSEEFEDYMEDFLILGDSLELE LLGSKRILDGIRSIDFERVGFEWEFVPLTVKMGALFEGRNL VQNIIVKLETKDMKVFLAGLEGYEKISDVLGNLFLHRFRTG EHLLGSEISVILQELCIDRSILLIPLSLLFDWFAFKDCRLC FSKSRSTLMYETVGGRFRLKGRSCDDWLGGSVAEDID

SEQ ID NO:	Description	Sequence
24	amino acid sequence of the Z protein of the Clone 13 strain of LCMV (GenBank Accession No. ABC96003.1; GI:86440168)	MGQGKSREEKGTNSTNRAEILPDTTYLGPLSCKSCWQKFDS LVRCHDHYLCRHCLNLLLSVSDRCPLCKYPLPTRLKISTAF SSPPPYEE
25	amino acid sequence of the GP protein of the WE strain of LCMV	MGQIVTMFEALPHIIDEVINIVIIVLIIITSIKAVYNFATC GILALVSFLFLAGRSCGMYGLNGPDIYKGVYQFKSVEFDMS HLNLTMPNACSANNSHHYISMGSSGLELTFTNDSIINHNFC NLTSAFNKKTFDHTLMSIVSSLHLSIRGNSNHKAVSCDFNN GITIQYNLSFSDPQSAISQCRTFRGRVLDMFRTAFGGKYMR SGWGWAGSDGKTTWCSQTSYQYLIIQNRTWENHCRYAGPFG MSRILPAQEKTKFLTPRLAGTFTWTLSDSSGVENPGGYCLT KWMILAAELKCFGNTAVAKCNVNHDEEFCDMLRLIDYNKAA LSKFKQDVESALHVFKTTVNSLISDQLLMRNHLRDLMGVPY CNYSKFWYLEHAKTGETSVPKCWLVTNGSYLNETHFSDQIE QEADNMITEMLPKDYIKRQGSTPLALMDLLMFSTSAYLISI FLHLVKIPTHRHIKGGSCPKPHRLTNKGICSCGAFKVPGVK TIWKRR
26	nucleotide sequence of the HBV HBe antigen (GenBank Accession No. E15688.1; GI: 5710371)	ATGGACATTGACACGTATAAAGAATTTGGAGCTACTGTGGA GTTACTCTCGTTTTTGCCTTCTGACTTCTTTCCTTCCGTCA GAGATCTCCTAGACACCGCCTCAGCTCTGTATCGAGAAGCC TTAGAGTCTCCTGAGCATTGCTCACCTCACCATACTGCACT
		CAGGCAAGCCATTCTCTGCTGGGGGGAATTGATGACTCTAG CTACCTGGGTGGGTAATAATTTGGAAGATCCAGCATCCAGG GATCTAGTAGTCAATTATGTTAATACTAACATGGGTTTAAA GATCAGGCAACTATTGTGGTTTCATATATCTTGCCTTACTT TTGGAAGAGACTGTACTTGAATATTTGGTCTCTTTCGGA GTGTGGATTCGCACTCCTCCAGCCTATAGACCACCAAATGC CCCTATCTTATCAACACTTCCGGAAACTACTGTTGTTTAA

7. EXAMPLES

7.1 Design of Arenavirus Vector Genome / Vector Construction

[0400] Based on established approaches (U.S. Patent Application Publication No. US 2010/0297172 A1; and Flatz L. et al., Nat Med. 2010 March; 16(3): 339-345), LCMV- and

Junin Virus (JUNV)-based vaccine vectors expressing the respective HBV antigens or certain domains thereof are designed (FIG. 1).

7.2 Vaccines Against Hepatitis B Virus

[0401] Candidate vaccines against hepatitis B virus (HBV) comprise rLCMV-based and rJUNV (Junin vaccine strain Candid#1) vectors expressing pre-S2/S (rLCMV/pre-S2/S, rJUNV/Pre-S2/S), HBc (rLCMV/HBc, rJUNV/HBc), a fusion protein consisting of the full length HBs and HBc ORFs (rLCMV/HBsHBc), and HBe (rLCMV/HBe, rJUNV/HBe). Vectors will be replication-deficient (r2LCMV, also referred to as rLCMV, r2JUNV, also referred to as rJUNV) and replication-competent trisegmented constructs (r3LCMV, r3JUNV; *see, e.g.,* Emonet et al., 2009, PNAS, 106(9):3473-3478), wherein the transgenes are arranged in a so-called "artificial" way (r3LCMV^{art}, r3JUNV^{art}). Mice (e.g., C57BL/6 mice) are immunized with one of these constructs, or with combinations thereof in a homologous or heterologous prime-boost vaccination. Administration is performed via the intraperitoneal, intramuscular, or intravenous route. The dose will be in the range of 10^4 to 10^7 focus forming units (FFU). At time points ranging from 7 to 100 days after immunization, HBV-specific CD8+ T cells are measured in the blood and/or spleen. T cells may be measured, for example, by using MHC class I tetramers in combination with anti-CD8 antibodies in order to identify the magnitude of the CD8+ T cell response to HBV-derived epitopes.

[0402] In a complementary approach, synthetic peptides are used to selectively stimulate directly ex vivo blood and/or spleen-derived CD8+ T cells by means of intracellular cytokine assays. The intracellular cytokine assays measure the frequency of interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and/or interleukin (IL)-2-producing CD8+ T cells. Surface expression of CD107a serves as a marker of cytolytic degranulation in flow cytometry (FACS). Peptide specificities are analyzed, including: HBs-derived epitope VWLSVIWM (SEQ ID NO: 8), HBs-derived epitope IPQSLDSWWTSL (SEQ ID NO: 9), and HBc-derived epitope MGLKFRQL (SEQ ID NO: 10).

7.3 Immunogenicity of Replication-Deficient Arenavirus-Based Vectors Expressing HBV Antigens

[0403] C57BL/6 mice (5 mice per group) were immunized once with 10^5 FFU of rLCMV/HBs-HBc (group 1), rLCMV/HBc (group 3), rLCMV/Pre-S2 (group 4), or with

10^4 FFU of rLCMV/HBs-HBc (group 2), via the intravenous route. Control mice were left untreated. 10 days after immunization CD8+ T cells were measured in the blood by using MHC class I multimers. H-$2K^b$ dextramers complexed with the HBs-derived epitope VWLSVIWM and H-$2K^b$ dextramers complexed with the HBc-derived epitope MGLKFRQL were used in combination with anti-CD8α antibody to identify hepatitis B virus-specific CD8+ T cells. The enumerated cells were expressed as a percentage of the total $CD8^+B220^-$ T cell pool in peripheral blood.

[0404] The results, as shown in Figure 3, indicate that vaccination with rLCMV/HBs-HBc, rLCMV/HBc and rLCMV/Pre-S2 induces substantial antigen-specific CD8+ T cell responses against the antigens expressed by the respective vectors. The anti-HBs and anti-HBc CD8+ T cell responses induced by vaccination with rLCMV/HBs-HBc showed a clear dose dependency. Higher frequencies of anti-HBc CD8+ T cells upon rLCMV/HBs-HBc immunization as compared to rLCMV/HBc immunization indicate that fusion to HBs results in augmented immunogenicity of HBc.

[0405] Anti-HBs CD8+ T cell frequencies were somewhat higher after immunization with rLCMV/Pre-S2 than after immunization with rLCMV/HBs-HBc, raising the possibility that anti-HBc CD8+ T cell responses competed with anti-HBs responses for antigen availability.

7.4 Immunogenicity of Attenuated Replication-Competent Arenavirus-Based Vectors Expressing HBV Antigens

[0406] C57BL/6 mice (5 mice per group) were immunized once with 10^5 FFU of r3LCMV/HBs-HBc (group 1), r3LCMV/HBc (group 2), r3LCMV/Pre-S2 (group 3), or with 10^5 FFU of rLCMV/HBs-HBc (group 4), via the intravenous route. Control mice were left without vaccination. 8 days after immunization HBs- and HBc-epitope-specific CD8+ T cells were measured in the blood by using MHC class I multimers. H-$2K^b$ dextramers complexed with the HBs-derived epitope VWLSVIWM and H-$2K^b$ dextramers complexed with the HBc-derived epitope MGLKFRQL were used in combination with anti-CD8α antibody to identify hepatitis B virus-specific CD8+ T cells. The enumerated cells were expressed in two different ways, either as a percentage of the total $CD8^+B220^-$ T cell pool in peripheral blood (FIG. 4A) or as a percentage of circulating lymphocytes in blood (FIG. 4B).

[0407] The results, as shown in Figure 4, indicate that all r3LCMV-based constructs as well as the replication-deficient rLCMV/HBs-HBc reference vector were immunogenic,

eliciting epitope-specific CD8+ T cells against their vectorized antigens, respectively. Moreover, when enumerating epitope-specific CD8+ T cells as a percentage of circulating lymphocytes, the replicating r3LCMV/HBs-HBc is shown to be more immunogenic than its replication-deficient counterpart rLCMV/HBs-HBc.

VACCINER MOD HEPATITIS B-VIRUS

PATENTKRAV

1. Infektiøs arenavirus-virusvektor, hvor en arenavirus-åben læseramme fjernes og erstattes af en nukleotidsekvens, der er valgt fra gruppen bestående af:
   a. en nukleotidsekvens, der koder for et HBV-HBc-protein eller et antigenfragment deraf,
   b. en nukleotidsekvens, der koder for en fusion af HBV-HBs- og HBc-proteiner eller antigenfragmenter deraf, og
   c. en nukleotidsekvens, der koder for et HBV-præ-S2/S-protein eller et antigenfragment deraf,

   hvor virusvektoren er i stand til at fremkalde et T-celle respons mod HBV-HBc-proteinet, fusionen af HBV-HBs- og HBc-proteiner, HBV-præ-S2/S-proteinet eller et antigenfragment deraf.

2. Virusvektor ifølge krav 1, hvor
   (i) HBc-proteinet eller antigenfragmentet deraf omfatter en aminosyresekvens, der er 80 %, 81 %, 82 %, 83 %, 84 %, 85 %, 86 %, 87 %, 88 %, 89 %, 90 %, 91 % 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % eller 100 % identisk med en aminosyresekvens, der kodes for af nukleotidsekvensen ifølge SEQ ID NO: 2,
   (ii) fusionen af HBV-HBs- og HBc-proteiner eller antigenfragmenter deraf omfatter en aminosyresekvens, der er 80 %, 81 %, 82 %, 83 %, 84 %, 85 %, 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % eller 100 % identisk med en aminosyresekvens, der kodes for af nukleotidsekvensen ifølge SEQ ID NO: 3, eller
   (iii) præ-S2/S-proteinet eller antigenfragmentet deraf omfatter en aminosyresekvens, der er 80 %, 81 %, 82 %, 83 %, 84 %, 85 %, 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % eller 100 % identisk med en aminosyresekvens, der kodes for af nukleotidsekvensen ifølge SEQ ID NO: 1.

3. Virusvektor ifølge krav 1, der omfatter mindst to eller mindst tre af:
   a. en nukleotidsekvens, der koder for et HBV-HBc-protein eller et

antigenfragment deraf,

b. en nukleotidsekvens, der koder for en fusion af HBV-HBs- og HBc-proteiner eller antigenfragmenter deraf, og

c. en nukleotidsekvens, der koder for et HBV-præ-S2/S-protein eller et antigenfragment deraf,

eventuelt hvor ekspression af nukleotidsekvenserne producerer et antigent proteinkompleks, der fremkalder højere titere af neutraliserende antistoffer end ekspression af de individuelle proteinkomplekskomponenter.

4. Virusvektor ifølge et hvilket som helst af kravene 1 til 3, hvor arenaviruset er:
   (i) lymfocytisk choriomeningitis-virus,
   (ii) replikationsdeficient og konstrueret til at indeholde et genom med evnen til at amplificere og udtrykke dets genetiske information i inficerede celler, men ude af stand til at producere yderligere infektiøse afkomspartikler i normale, ikke gensplejsede celler,
   (iii) bisegmenteret og replikationsdeficient eller
   (iv) trisegmenteret og replikationskompetent.

5. Virusvektor ifølge et hvilket som helst af kravene 1 til 4, hvor den åbne læseramme, der koder for glycoproteinet (GP) af arenaviruset, er deleteret eller funktionelt inaktiveret.

6. Virusvektor ifølge et hvilket som helst af kravene 1 til 5, hvor den genomiske information, der koder for den infektiøse arenavirus-virusvektor, er afledt af lymfocytisk choriomeningitis-virus eller Pichinde-virus,
   hvor den lymfocytiske choriomeningitis-virus eventuelt er den lymfocytiske choriomeningitis-virus-klon 13-stamme eller den lymfocytiske choriomeningitis-virus-MP-stamme.

7. Virusvektor ifølge et hvilket som helst af kravene 1 til 6, hvor virusvektoren omfatter et genomisk segment, hvor det genomiske segment omfatter:
   (i) en nukleotidsekvens, der er mindst 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, mindst 99 % eller 100 % identisk med sekvensen af nukleotiderne 1639 til 3315 ifølge SEQ ID NO: 11 eller nukleotiderne 1640 til 3316 ifølge SEQ ID NO: 12, eller

(ii) en nukleotidsekvens, der koder for et ekspressionsprodukt, hvis aminosyresekvens er mindst 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, mindst 99 % eller 100 % identisk med aminosyresekvensen, der kodes for af nukleotiderne 1639 til 3315 ifølge SEQ ID NO: 11 eller nukleotiderne 1640 til 3316 ifølge SEQ ID NO: 12.

8. Virusvektor ifølge et hvilket som helst af kravene 1 til 7, hvor arenavirusets vækst eller infektivitet ikke påvirkes af nukleotidsekvensen.

9. Virusvektor ifølge et hvilket som helst af kravene 1 til 8, hvor arenaviruset er en infektiøs, replikationsdeficient arenavirus-virusvektor, der er konstrueret til at indeholde et genom med evnen til at amplificere og udtrykke dets genetiske information i inficerede celler, men ude af stand til at producere yderligere infektiøse afkomspartikler i normale, ikke gensplejsede celler, og hvor indgivelsen af arenavirus-virusvektoren til et individ inducerer et langvarigt immunrespons mod HBV-HBc-proteinet, fusionen af HBV-HBs- og HBc-proteiner, HBV-præ-S2/S-proteinet eller et antigenfragment deraf, eventuelt hvor

(i) det langvarige immunrespons inducerer en detekterbar antistoftiter mod HBV-HBc-proteinet, fusionen af HBV-HBs- og HBc-proteiner, HBV-præ-S2/S-proteinet eller et antigenfragment deraf, eller

(ii) det langvarige immunrespons inducerer en detekterbar antistoftiter mod HBV-HBc-proteinet, fusionen af HBV-HBs- og HBc-proteiner, HBV-præ-S2/S-proteinet eller et antigenfragment deraf i mindst et minimum af 4 uger,

eventuelt hvor det langvarige immunrespons øger antistoftiteren mod HBV-HBc-proteinet, fusionen af HBV-HBs- og HBc-proteiner, HBV-præ-S2/S-proteinet eller et antigenfragment deraf med mindst 100 %, mindst 200 %, mindst 300 %, mindst 400 %, mindst 500 % eller mindst 1000 %.

10. Farmaceutisk sammensætning, immunogen sammensætning eller vaccine, der omfatter virusvektoren ifølge et hvilket som helst af kravene 1 til 9 og en farmaceutisk acceptabel bærer.

11. Virusvektor som defineret i et hvilket som helst af kravene 1 til 9, eller farmaceutisk sammensætning, immunogen sammensætning eller vaccine som defineret i krav 10, til anvendelse i en fremgangsmåde til behandling eller forebyggelse af en

hepatitis B-virusinfektion hos en patient.

12. Isoleret nukleinsyre, hvor nukleinsyren omfatter et genomisk arenavirussegment, hvor en åben læseramme af det genomiske segment er deleteret eller funktionelt inaktiveret, og hvor det genomiske segment omfatter en eller flere af:
    a. en nukleotidsekvens, der koder for et HBV-HBc-protein eller et antigenfragment deraf,
    b. en nukleotidsekvens, der koder for en fusion af HBV-HBs- og HBc-proteiner eller antigenfragmenter deraf, eller
    c. en nukleotidsekvens, der koder for et HBV-præ-S2/S-protein eller et antigenfragment deraf,

    hvor en en virusvektor, der omfatter det genomiske arenavirussegment, er i stand til at fremkalde et T-cellerespons mod HBV-HBc-proteinet, fusionen af HBV-HBs- og HBc-proteiner, HBV-præ-S2/S-proteinet eller et antigenfragment deraf,
    eventuelt hvor det genomiske segment er det korte segment, hvor den åbne læseramme, der koder for glycoproteinet (GP), er deleteret.

13. cDNA af det genomiske arenavirussegment som defineret i krav 12.

14. *In vitro*-fremgangsmåde til generering af en infektiøs, replikationsdeficient arenavirusvektor, der omfatter:
    a. transfektion i en værtscelle af cDNA'et ifølge krav 13,
    b. opretholdelse af værtscellen under betingelser, der er egnede til virusdannelse, og
    c. høst af den infektiøse, replikationsdeficiente arenavirusvektor,

    hvor værtscellen udtrykker den åbne læseramme af det genomiske segment, der er deleteret eller funktionelt inaktiveret,
    eventuelt hvor fremgangsmåden yderligere omfatter i trin a. transfektion i værtscellen af: et cDNA af et andet genomisk arenavirussegment, en nukleinsyre, der omfatter L-protein ORF og/eller en nukleinsyre, der omfatter NP ORF.

15. Farmaceutisk sammensætning, der omfatter en første infektiøs, replikationsdeficient arenavirus-virusvektor, der er konstrueret til at indeholde et genom med evnen til at amplificere og udtrykke dets genetiske information i inficerede celler, men ude af stand til at producere yderligere infektiøse

afkomspartikler i normale, ikke gensplejsede celler, hvor en arenavirus-åben læseramme fjernes og erstattes af en første nukleotidsekvens, der er valgt fra gruppen bestående af:

a. en nukleotidsekvens, der koder for et HBV-HBc-protein eller et antigenfragment deraf,
b. en nukleotidsekvens, der koder for en fusion af HBV-HBs- og HBc-proteiner eller antigenfragmenter deraf, og
c. en nukleotidsekvens, der koder for et HBV-præ-S2/S-protein eller et antigenfragment deraf,

og en anden infektiøs, replikationsdeficient arenavirus-virusvektor, der er konstrueret til at indeholde et genom med evnen til at amplificere og udtrykke dets genetiske information i inficerede celler, men ude af stand til at producere yderligere infektiøse afkomspartikler i normale, ikke gensplejsede celler, hvor en arenavirus-åben læseramme fjernes og erstattes af en anden nukleotidsekvens, der er valgt fra gruppen bestående af:

a. en nukleotidsekvens, der koder for et HBV-HBc-protein eller et antigenfragment deraf,
b. en nukleotidsekvens, der koder for en fusion af HBV-HBs- og HBc-proteiner eller antigenfragmenter deraf, og
c. en nukleotidsekvens, der koder for et HBV-præ-S2/S-protein eller et antigenfragment deraf,

hvor den første og anden nukleotidsekvens er forskellige, hvor den første virusvektor og den anden virusvektor er i stand til at fremkalde et T-cellerespons mod HBV-HBc-proteinet, fusionen af HBV-HBs- og HBc-proteiner, HBV-præ-S2/S-proteinet eller et antigenfragment deraf.

16. Virusvektor ifølge et hvilket som helst af kravene 1 til 9, eller farmaceutisk sammensætning, immunogen sammensætning eller vaccine ifølge krav 10, eller farmaceutisk sammensætning ifølge krav 15, hvor virusvektoren, den farmaceutiske sammensætning, den immunogene sammensætning eller vaccinen er egnet til intramuskulær indgivelse eller intravenøs indgivelse.

DRAWINGS

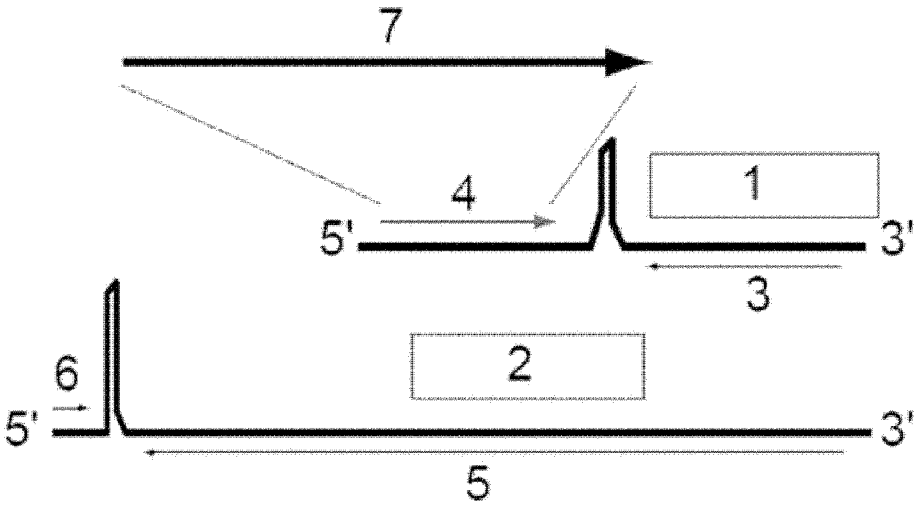
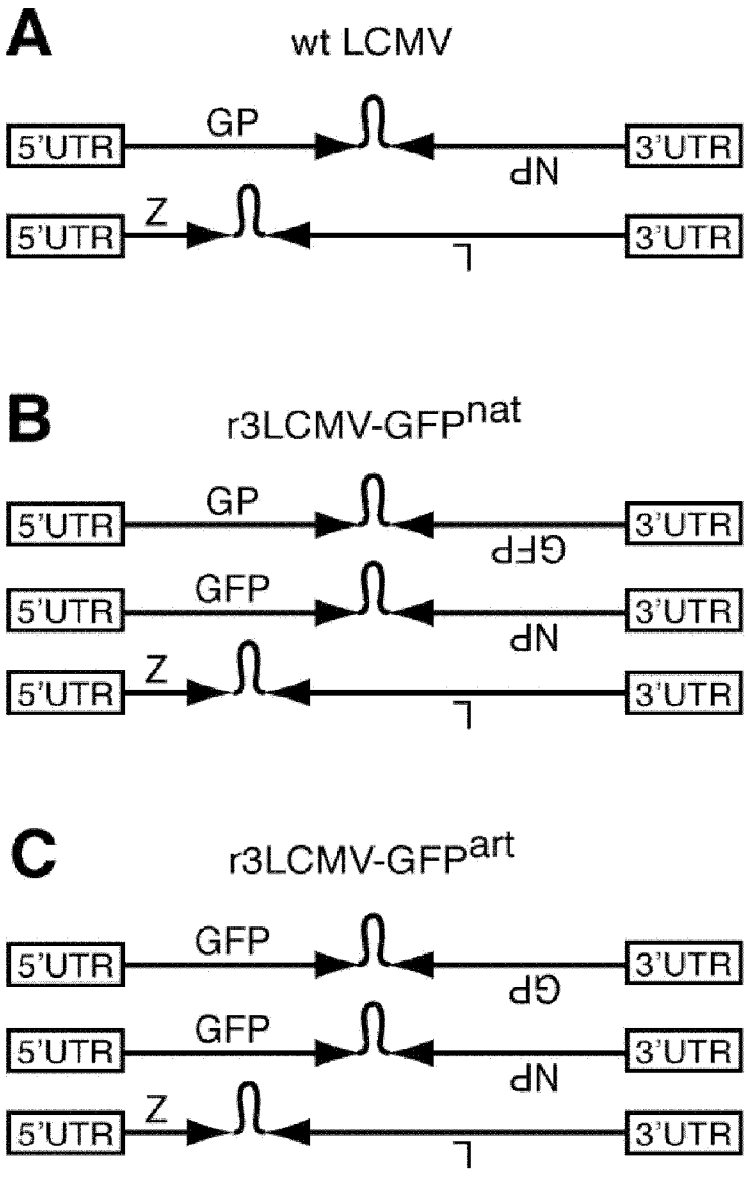


Fig. 1


A
wt LCMV
5'UTR
GP
3'UTR
NP
5'UTR
Z
3'UTR
L
B
r3LCMV-GFP^nat
5'UTR
GP
3'UTR
GFP
5'UTR
GFP
3'UTR
NP
5'UTR
Z
3'UTR
L
C
r3LCMV-GFP^art
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3'UTR
GP
5'UTR
GFP
3'UTR
NP
5'UTR
Z
3'UTR
L

Fig. 2A- 2C

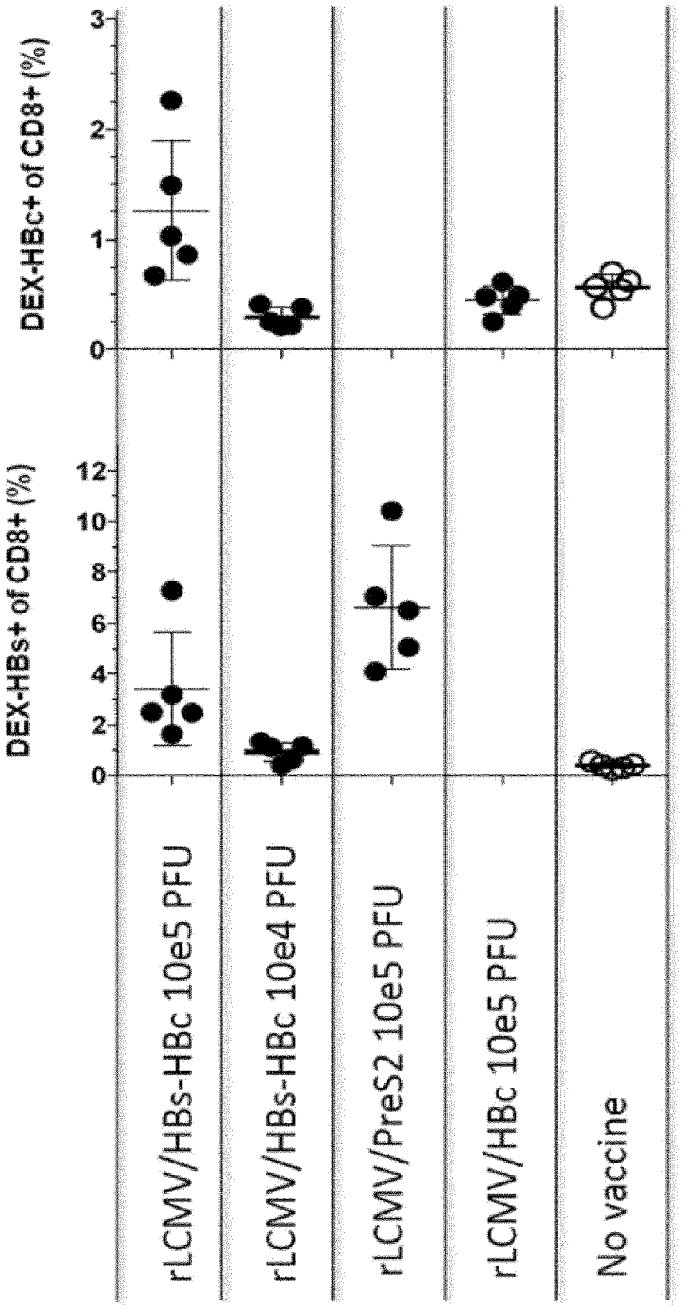
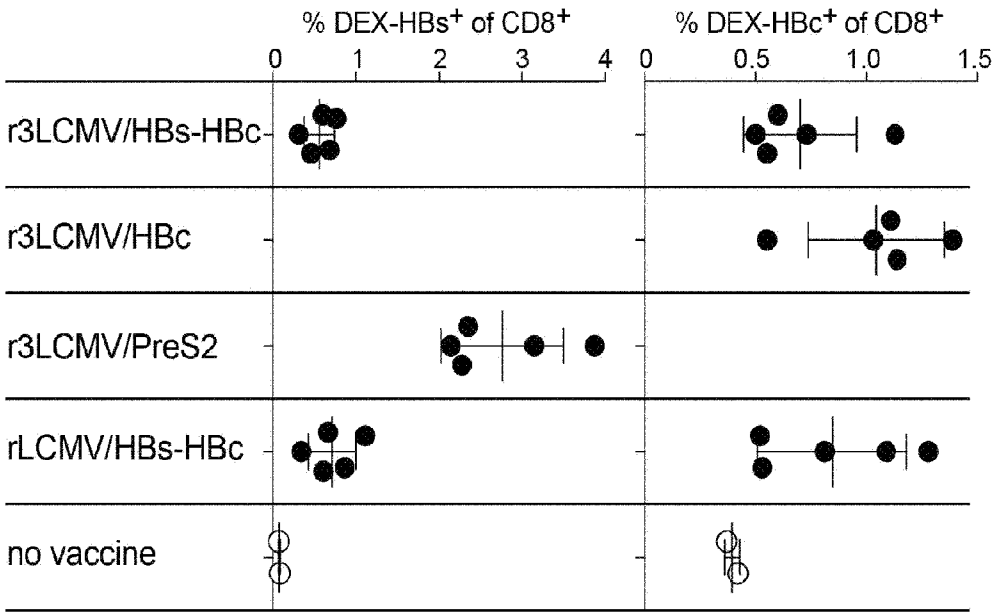

DEX-HBs+ of CD8+ (%)
DEX-HBc+ of CD8+ (%)
0
2
4
6
8
10
12
0
1
2
3
rLCMV/HBs-HBc 10e5 PFU
rLCMV/HBs-HBc 10e4 PFU
rLCMV/PreS2 10e5 PFU
rLCMV/HBc 10e5 PFU
No vaccine

Fig. 3

A


% DEX-HBs+ of CD8+
0
1
2
3
4
% DEX-HBc+ of CD8+
0
0.5
1.0
1.5
r3LCMV/HBs-HBc
r3LCMV/HBc
r3LCMV/PreS2
rLCMV/HBs-HBc
no vaccine

B

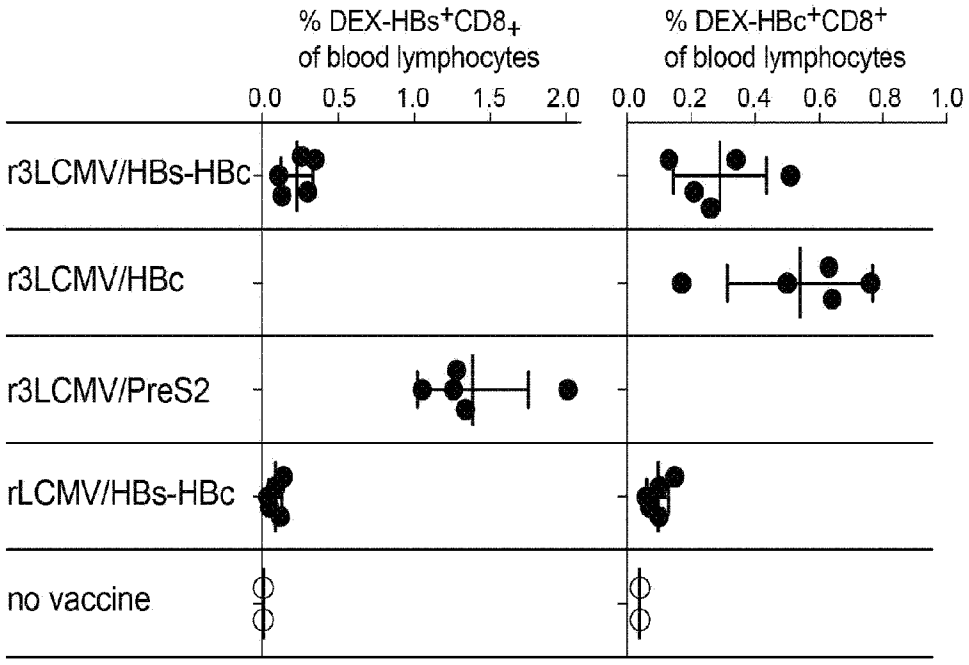

% DEX-HBs+CD8+
of blood lymphocytes
0.0
0.5
1.0
1.5
2.0
% DEX-HBc+CD8+
of blood lymphocytes
0.0
0.2
0.4
0.6
0.8
1.0
r3LCMV/HBs-HBc
r3LCMV/HBc
r3LCMV/PreS2
rLCMV/HBs-HBc
no vaccine

Fig. 4A - 4B