Title: METHODS AND COMPOSITIONS FOR ELICITING AN IMMUNE RESPONSE AGAINST HEPATITIS B VIRUS

Abstract: The present invention relates to immunization of hypo-responsive groups of individuals. In particular, the present invention provides methods and compositions for eliciting a potent immune response to hepatitis B virus in individuals in need there-of.
METHODS AND COMPOSITIONS FOR ELICITING AN IMMUNE RESPONSE AGAINST HEPATITIS B VIRUS

FIELD

[0001] The present invention relates to immunization of hypo-responsive groups of individuals. In particular, the present invention provides methods and compositions for eliciting a potent immune response to hepatitis B virus in individuals in need thereof.

BACKGROUND

[0002] Hepatitis B virus (HBV) is one of several viruses known to cause liver disease (e.g., cirrhosis, liver failure and hepatocellular carcinoma) in humans. HBV is spread through percutaneous or mucosal contact with infected body fluids. According to the World Health Organization (WHO), nearly two billion people are infected with HBV, which causes over 600,000 fatalities each year (WHO, Fact Sheet No. 204, 2009).

[0003] The HBV virion is composed of a core antigen (HBcAg), which encapsulates viral DNA, and a surface antigen (HBsAg), which is located on the viral outer membrane. HBsAg, previously known as the Australian antigen, is composed of three glycoproteins having a shared carboxy terminal sequence: S (S only); M (pre-S2 and S); and L (pre-S1, pre-S2 and S). HBsAg self-associates to form 22 nm particles that are released from infected hepatocytes.

[0004] All HBV vaccines approved for use in humans are based on noninfectious HBsAg particles. In the United States, the current HBV vaccines are recombinant subunit vaccines produced in yeast (e.g., RECOMBIVAX HB® hepatitis B vaccine marketed by Merck & Co.; and ENGERIX-B® hepatitis B vaccine marketed by GlaxoSmithKline). These HBV vaccines are formulated as HBsAg adsorbed to alum.

[0005] Use of the current vaccines is hindered by the typical lengthy administration regimen (e.g., generally three doses over six-months). In addition, only 10-20% of adult vaccine recipients mount a seroprotective immune response within one month of receiving a first dose of a HBV vaccine (Andre and Sarary, Post Grad Med J, 63: 169-178, 1987; and Keating and Noble, Drugs 2003, 63:1021-1051, 2003). This delay in the generation of a protective antibody response is of particular importance for individuals at high risk of HBV infection (e.g., health care workers or individuals in high risk behavior groups). Further, compliance in returning for three injections can be poor, especially since visits are spaced over such a long period of time.

[0006] Another serious shortcoming of the current HBV vaccines is the high level of hypo- or non-responders (30-60% hypo- or nonresponders after the recommended regimen) among some groups, such as those over 50 years of age (Denis et al., J Infect Dis, 149:1019, 1984), and subjects with renal failure or diabetes (Weber et al., JAMA, 254:3187-3189, 1985). Thus, development of a more potent HBV vaccine with more rapid induction of protective immunity,
and improved response among hypo-responder populations is of major importance in reducing HBV infection.

SUMMARY

[0007] The present invention relates to immunization of hypo-responsive groups of individuals. In particular, the present invention provides methods and compositions for eliciting a potent immune response to hepatitis B virus in individuals in need thereof.

[0008] Specifically, the present disclosure provides methods for eliciting an immune response against hepatitis B virus (HBV) in a human subject having a glucose metabolism disorder, comprising: administering to a human subject a first and a second dose of an immunogenic composition on at least two separate occasions, wherein the immunogenic composition comprises a hepatitis B surface antigen (HBsAg), and an immunostimulatory sequence (ISS) of from 8 to 50 nucleotides in length comprising an unmethylated cytosine-phosphate-guanosine (CpG) motif, wherein the subject has a glucose metabolism disorder selected from the group consisting of type I diabetes, type II diabetes, and pre-diabetes at the onset of the administering; and wherein the HBsAg and the ISS are present in the immunogenic composition in amounts effective to elicit an immune response in the subject at least two months after the second dose. In some embodiments, the methods further comprise administering a subunit vaccine comprising HBsAg adsorbed to alum as a subsequent dose (e.g., a booster at least 6 months after the first dose, preferably at least 1 year, 5 years or 10 years after the first dose). In some embodiments, the ISS comprises the nucleotide sequence 5’-TCG-3’. In some embodiments, the ISS comprises the nucleotide sequence ‘5-CGTTCTG-3’ or ‘5-AACGTTCG-3’. In some embodiments, the ISS comprises the nucleotide sequence of SEQ ID NO:1. In other embodiments, the ISS comprises the nucleotide sequence of SEQ ID NO:2. In some preferred embodiments, the ISS comprises a phosphate backbone modification. In some preferred embodiments, the ISS comprises a phosphorothioate backbone modification. In some preferred embodiments, the ISS is 1018 ISS. In some embodiments, the immunogenic composition comprises 20 μg or less of the HBsAg. In some embodiments, the immunogenic composition comprises 3000 μg or less of the ISS. In some embodiments, the immunogenic composition further comprises a buffer. In some embodiments, the buffer comprises sodium phosphate and sodium chloride. In some preferred embodiments, the immunogenic composition is buffered from pH 6.5 to 7.5, or about to about pH 7.0. In some embodiments, the immunogenic composition further comprises a surfactant. In a subset of these embodiments, the surfactant comprises polysorbate. In some embodiments, the immunogenic composition further comprises an additional adjuvant. In a subset of these embodiments, the additional adjuvant is comprises alum. In some preferred embodiments, the HBsAg is a recombinant HBsAg produced in yeast. In some preferred embodiments, the immune response is
a seroprotective antibody response comprising an anti-HBsAg response of at least 10 mIU/mL at least two months after the second dose. In a subset of these embodiments, the anti-HBsAg response is at least 15, 20, or 25 mIU/mL at least two months after the second dose. In some embodiments, the immune response is a seroprotective antibody response comprising an anti-HBsAg response of at least 10 mIU/mL at least six months after the second dose. In a subset of these embodiments, the anti-HBsAg response is at least 20, 30, 40 or 50 mIU/mL at least six months after the second dose. In some preferred embodiments, the seroprotective antibody response is statistically greater than that elicited by administration of a control immunogenic composition lacking the ISS. In some preferred embodiments, the subject has type II diabetes. In some embodiments, the subject is taking one or both of an oral hypoglycemic and insulin, at the onset of the administering. In some embodiments, the oral hypoglycemic comprises one or more of the group consisting of a biguanide, a sulfonylurea, a nonsulfonylurea secretagogue, an alpha glucosidase inhibitor, and a thiazolidinedione. In some embodiments, the biguanide is metformin. In some embodiments, the insulin is recombinant human insulin or an analog thereof. In some preferred embodiments, the subject has a body mass index of greater than 25 (overweight). In other preferred embodiments, the subject has a body mass index of greater than 30 (obese), or a body mass index of greater than 40 (morbidly obese). In some embodiments, the subject is a resident of a nursing home or an assisted living facility. In other embodiments, the subject is a resident of a correctional facility. In some embodiments, the subject is over 40 years of age. In a subset of these embodiments, the subject is from 41 to 60 or 61 to 80 years of age. In some preferred embodiments, the HBsAg comprises the S antigen. In other preferred embodiments, the HBsAg further comprises one or both of the pre-S2 antigen, and the pre-S1 antigen. In some embodiments, the HBsAg antigen is purified from plasma of an HBV-infected subject. In other embodiments, the HBsAg antigen is a recombinant HBsAg produced in mammalian cells in vitro. In further embodiments, the present disclosure provides methods for eliciting an immune response against hepatitis B virus (HBV) in a human subject having a glucose metabolism disorder, comprising: administering to a human subject an effective amount of an immunogenic composition, wherein the immunogenic composition comprises a hepatitis B surface antigen (HBsAg), and an immunostimulatory sequence (ISS) of from 8 to 50 nucleotides in length comprising an unmethylated cytosine-phosphate-guanosine (CpG) motif, and wherein the subject has a glucose metabolism disorder selected from the group consisting of type I diabetes, type II diabetes, and pre-diabetes at the onset of the administering. In some embodiments, the immunogenic composition is administered on at least two separate occasions as a first dose and a second dose, and the effective amount is sufficient to elicit an immune response in the subject after the second dose. Also provides are kits for eliciting an immune response
against hepatitis B virus (HBV) in a human subject having a glucose metabolism disorder, comprising: an immunogenic composition comprising a hepatitis B surface antigen (HBsAg), and an immunostimulatory sequence (ISS) of from 8 to 50 nucleotides in length comprising an unmethylated cytosine-phosphate-guanosine (CpG) motif; and instructions for administering to a human subject an effective amount of the immunogenic composition, wherein the subject has a glucose metabolism disorder selected from the group consisting of type I diabetes, type II diabetes, and pre-diabetes.

[0009] Additionally, the present disclosure provides immunogenic compositions comprising an immunostimulatory sequence (ISS) and a hepatitis B surface antigen (HBsAg) for use in eliciting an immune response against hepatitis B virus (HBV) in a human subject when administered as a first dose and a second dose on at least two separate occasions, wherein the ISS is from 8 to 50 nucleotides in length comprising an unmethylated cytosine-phosphate-guanosine (CpG) motif, wherein the ISS and the HBsAg are present in the immunogenic composition in amounts effective to elicit an immune response in the subject at least two months after the second dose, and wherein the subject has a glucose metabolism disorder selected from the group consisting of type I diabetes, type II diabetes, and pre-diabetes at the onset of the administering. Also provided by the present disclosure are immunogenic compositions comprising a immunostimulatory sequence (ISS) and a hepatitis B surface antigen (HBsAg) for use in preventing a human subject from being infected with a hepatitis B virus (HBV) when administered as a first dose and a second dose on at least two separate occasions, wherein the ISS is from 8 to 50 nucleotides in length comprising an unmethylated cytosine-phosphate-guanosine (CpG) motif, wherein the ISS and the HBsAg are present in the immunogenic composition in amounts effective to prevent the subject from becoming infected with HBV at least two months after the second dose, and wherein the subject has a glucose metabolism disorder selected from the group consisting of type I diabetes, type II diabetes, and pre-diabetes at the onset of the administering. In some embodiments, the ISS comprises the nucleotide sequence 5’-TCG-3’. In some embodiments, the ISS comprises the nucleotide sequence ‘5-CGTTCCG-3’ or ‘5-AACGTTCC-3’. In some embodiments, the ISS comprises the nucleotide sequence of SEQ ID NO:1. In other embodiments, the ISS comprises the nucleotide sequence of SEQ ID NO:2. In some preferred embodiments, the ISS comprises a phosphate backbone modification. In some preferred embodiments, the ISS comprises a phosphorothioate backbone modification. In some preferred embodiments, the ISS is 1018 ISS. In some embodiments, the immunogenic composition comprises 20 µg or less of the HBsAg. In some embodiments, the immunogenic composition comprises 3000 µg or less of the ISS. In some embodiments, the immunogenic composition further comprises a buffer. In some embodiments, the buffer comprises sodium phosphate and
sodium chloride. In some preferred embodiments, the immunogenic composition is buffered from pH 6.5 to 7.5, or about to about pH 7.0. In some embodiments, the immunogenic composition further comprises a surfactant. In a subset of these embodiments, the surfactant comprises polysorbate. In some embodiments, the immunogenic composition further comprises an additional adjuvant. In a subset of these embodiments, the additional adjuvant is comprises alum. In some preferred embodiments, the HBsAg is a recombinant HBsAg produced in yeast. In some preferred embodiments, the immune response is a seroprotective antibody response comprising an anti-HBsAg response of at least 10 mIU/mL at least two months after the second dose. In a subset of these embodiments, the anti-HBsAg response is at least 15, 20, or 25 mIU/mL at least two months after the second dose. In some embodiments, the immune response is a seroprotective antibody response comprising an anti-HBsAg response of at least 10 mIU/mL at least six months after the second dose. In a subset of these embodiments, the anti-HBsAg response is at least 20, 30, 40 or 50 mIU/mL at least six months after the second dose. In some preferred embodiments, the seroprotective antibody response is statistically greater than that elicited by administration of a control immunogenic composition lacking the ISS. In some preferred embodiments, the subject has type II diabetes. In some embodiments, the subject is taking one or both of an oral hypoglycemic and insulin, at the onset of the administering. In some embodiments, the oral hypoglycemic comprises one or more of the group consisting of a biguanide, a sulfonylurea, a nonsulfonylurea secretagogue, an alpha glucosidase inhibitor, and a thiazolidinedione. In some embodiments, the biguanide is metformin. In some embodiments, the insulin is recombinant human insulin or an analog thereof. In some preferred embodiments, the subject has a body mass index of greater than 25 (overweight). In other preferred embodiments, the subject has a body mass index of greater than 30 (obese), or a body mass index of greater than 40 (morbidly obese). In some embodiments, the subject is a resident of a nursing home or an assisted living facility. In other embodiments, the subject is a resident of a correctional facility. In some embodiments, the subject is over 40 years of age. In a subset of these embodiments, the subject is from 41 to 60 or 61 to 80 years of age. In some preferred embodiments, the HBsAg comprises the S antigen. In other preferred embodiments, the HBsAg further comprises one or both of the pre-S2 antigen, and the pre-S1 antigen. In some embodiments, the HBsAg antigen is purified from plasma of an HBV-infected subject. In other embodiments, the HBsAg antigen is a recombinant HBsAg produced in mammalian cells in vitro.

[0010] Moreover, the present disclosure provides immunogenic compositions comprising an immunostimulatory sequence (ISS) and a hepatitis B surface antigen (HBsAg) for use in preparing a medicament to elicit an immune response against hepatitis B virus (HBV) in a human subject when administered as a first dose and a second dose on at least two separate occasions,
wherein the ISS is from 8 to 50 nucleotides in length comprising an unmethylated cytosine-phosphate-guanosine (CpG) motif, wherein the ISS and the HBsAg are present in the immunogenic composition in amounts effective to elicit an immune response in the subject at least two months after the second dose, and wherein the subject has a glucose metabolism disorder selected from the group consisting of type I diabetes, type II diabetes, and pre-diabetes at the onset of the administering. Also provided by the present disclosure are immunogenic compositions comprising an immunostimulatory sequence (ISS) and a hepatitis B surface antigen (HBsAg) for use in preparing a medicament for preventing a human subject from being infected with a hepatitis B virus (HBV) when administered as a first dose and a second dose on at least two separate occasions, wherein the ISS is from 8 to 50 nucleotides in length comprising an unmethylated cytosine-phosphate-guanosine (CpG) motif, wherein the ISS and the HBsAg are present in the immunogenic composition in amounts effective to prevent the subject from becoming infected with HBV at least two months after the second dose, and wherein the subject has a glucose metabolism disorder selected from the group consisting of type I diabetes, type II diabetes, and pre-diabetes at the onset of the administering. In some embodiments, the ISS comprises the nucleotide sequence 5’-TCG-3’. In some embodiments, the ISS comprises the nucleotide sequence ‘5-CGTTTCG-3’ or ‘5-AACGTTTCG-3’. In some embodiments, the ISS comprises the nucleotide sequence of SEQ ID NO:1. In other embodiments, the ISS comprises the nucleotide sequence of SEQ ID NO:2. In some preferred embodiments, the ISS comprises a phosphate backbone modification. In some preferred embodiments, the ISS comprises a phosphorothioate backbone modification. In some preferred embodiments, the ISS is 1018 ISS. In some embodiments, the immunogenic composition comprises 20 μg or less of the HBsAg. In some embodiments, the immunogenic composition comprises 3000 μg or less of the ISS. In some embodiments, the immunogenic composition further comprises a buffer. In some embodiments, the buffer comprises sodium phosphate and sodium chloride. In some preferred embodiments, the immunogenic composition is buffered from pH 6.5 to 7.5, or about to about pH 7.0. In some embodiments, the immunogenic composition further comprises a surfactant. In a subset of these embodiments, the surfactant comprises polysorbate. In some embodiments, the immunogenic composition further comprises an additional adjuvant. In a subset of these embodiments, the additional adjuvant is comprises alum. In some preferred embodiments, the HBsAg is a recombinant HBsAg produced in yeast. In some preferred embodiments, the immune response is a seroprotective antibody response comprising an anti-HBsAg response of at least 10 mIU/mL at least two months after the second dose. In a subset of these embodiments, the anti-HBsAg response is at least 15, 20, or 25 mIU/mL at least two months after the second dose. In some embodiments, the immune response is a seroprotective antibody response comprising an
anti-HBsAg response of at least 10 mIU/mL at least six months after the second dose. In a subset of these embodiments, the anti-HBsAg response is at least 20, 30, 40 or 50 mIU/mL at least six months after the second dose. In some preferred embodiments, the seroprotective antibody response is statistically greater than that elicited by administration of a control immunogenic composition lacking the ISS. In some preferred embodiments, the subject has type II diabetes. In some embodiments, the subject is taking one or both of an oral hypoglycemic and insulin, at the onset of the administering. In some embodiments, the oral hypoglycemic comprises one or more of the group consisting of a biguanide, a sulfonylurea, a nonsulfonylurea secretagogue, an alpha glucosidase inhibitor, and a thiazolidinedione. In some embodiments, the biguanide is metformin. In some embodiments, the insulin is recombinant human insulin or an analog thereof. In some preferred embodiments, the subject has a body mass index of greater than 25 (overweight). In other preferred embodiments, the subject has a body mass index of greater than 30 (obese), or a body mass index of greater than 40 (morbidly obese). In some embodiments, the subject is a resident of a nursing home or an assisted living facility. In other embodiments, the subject is a resident of a correctional facility. In some embodiments, the subject is over 40 years of age. In a subset of these embodiments, the subject is from 41 to 60 or 61 to 80 years of age. In some preferred embodiments, the HBsAg comprises the S antigen. In other preferred embodiments, the HBsAg further comprises one or both of the pre-S2 antigen, and the pre-S1 antigen. In some embodiments, the HBsAg antigen is purified from plasma of an HBV-infected subject. In other embodiments, the HBsAg antigen is a recombinant HBsAg produced in mammalian cells in vitro.

BRIEF DESCRIPTION OF DRAWINGS

[0011] Figure 1 depicts the level of seroprotection provided by immunization of diabetic subjects with HEPLISAV® or ENGERIX-B® respectively.

General Techniques

[0012] The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, Molecular Cloning: A Laboratory Manual, second edition (Sambrook et al., 1989); Oligonucleotide Synthesis (Gait, ed., 1984); Animal Cell Culture (Freshney, ed., 1987); Handbook of Experimental Immunology (Weir & Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (Miller & Calos, eds., 1987); Current Protocols in Molecular Biology (Ausubel et al., eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis et al., eds., 1994); Current Protocols in

Definitions

[0013] As used herein, the singular form “a”, “an”, and “the” includes plural references unless indicated otherwise. For example, “an” excipient includes one or more excipients.

[0014] The phrase “comprising” as used herein is open-ended, indicating that such embodiments may include additional elements. In contrast, the phrase “consisting of” is closed, indicating that such embodiments do not include additional elements (except for trace impurities). The phrase “consisting essentially of” is partially closed, indicating that such embodiments may further comprise elements that do not materially change the basic characteristics of such embodiments.

[0015] As used interchangeably herein, the terms “polynucleotide” and “oligonucleotide” include single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), single-stranded RNA (ssRNA) and double-stranded RNA (dsRNA), modified oligonucleotides and oligonucleosides or combinations thereof. The oligonucleotide can be linearly or circularly configured, or the oligonucleotide can contain both linear and circular segments. Oligonucleotides are polymers of nucleosides joined, generally, through phosphodiester linkages, although alternate linkages, such as phosphorothioate esters may also be used in oligonucleotides. A nucleoside consists of a purine (adenine (A) or guanine (G) or derivative thereof) or pyrimidine (thymine (T), cytosine (C) or uracil (U), or derivative thereof) base bonded to a sugar. The four nucleoside units (or bases) in DNA are called deoxyadenosine, deoxyguanosine, thymidine, and deoxycytidine. A nucleotide is a phosphate ester of a nucleoside.

[0016] The term “immunostimulatory sequence” or “ISS” as used herein refers to a CpG-containing oligonucleotide in which the C is unmethylated, and which contributes to a measurable immune response as measured in vitro, in vivo and/or ex vivo. Examples of measurable immune responses include, but are not limited to, antigen-specific antibody production, secretion of cytokines, activation or expansion of lymphocyte populations such as NK cells, CD4+ T lymphocytes, CD8+ T lymphocytes, B lymphocytes, and the like. Preferably, the ISS preferentially activates a Th1-type response.

[0017] An “effective amount” or a “sufficient amount” of a substance is that amount sufficient to effect beneficial or desired results, including clinical results, and, as such, an
“effective amount” depends upon the context in which it is being applied. In the context of administering an immunogenic composition, an effective amount contains sufficient ISS and HBsAg to elicit an immune response (preferably a seroprotective level of antibody to HBsAg or anti-HBsAg). An effective amount can be administered in one or more doses.

[0018] As used herein the term “immunization” refers to a process that increases an organisms’ reaction to antigen and therefore improves its ability to resist or overcome infection.

[0019] The term “vaccination” as used herein refers to the introduction of vaccine into a body of an organism.

[0020] “Adjuvant” refers to a substance which, when added to a composition comprising an antigen, nonspecifically enhances or potentiates an immune response to the antigen in the recipient upon exposure.

[0021] The condition of "hyperglycemia" (high blood sugar) is a condition in which the blood glucose level is too high. Typically, hyperglycemia occurs when the blood glucose level rises above 180 mg/dl. Symptoms of hyperglycemia include frequent urination, excessive thirst and, over a longer time span, weight loss.

[0022] On the other hand, "hypoglycemia" (low blood sugar) is a condition in which the blood glucose level is too low. Typically, hypoglycemia occurs when the blood glucose level falls below 70 mg/dl. Symptoms of hypoglycemia include moodiness, numbness of the extremities (especially in the hands and arms), confusion, shakiness or dizziness.

[0023] The term "impaired glucose tolerance" is used to describe a person who, when given a glucose tolerance test, has a blood glucose level that falls between normal and hyperglycemic. Such a person is at a higher risk of developing diabetes, although they are not considered to have diabetes.

[0024] The term "glucose non-responsive" as used herein describes both the complete inability of cells, or islets to respond to treatment with or administration of glucose, as well as decreased responsiveness to glucose (e.g., by cells that do not produce sufficient levels of insulin in response to glucose or that require significantly higher levels of glucose to respond at normal levels).

**DETAILED DESCRIPTION**

[0025] The present invention relates to immunization of hypo-responsive groups of individuals. In particular, the present invention provides methods and compositions for eliciting a potent immune response to hepatitis B virus in subjects having a glucose metabolism disorder.
HEPLISAV® (recombinant HBsAg + 1018 ISS vaccine developed by Dynavax Technologies Corporation) has been shown to produce a more rapid, higher titer, and sustained seroprotective antibody response in healthy adults as compared to a currently licensed hepatitis B vaccine (Barry and Cooper, Expert Opin Biol Ther, 7:1731-1737, 2007; Halperin et al., Vaccine, 21:2461-2467, 2003; Halperin et al., Vaccine, 24:20-26, 2006; Madaan, Drugs of the Future, 34:531-535, 2009; and Sung and Chan, Curr Opin Molec Ther, 8:150-155, 2006). Other CpG-containing oligodeoxynucleotides (ODN) have been shown to improve the immunogenicity of hepatitis B virus (HBV) vaccines in preclinical and clinical studies (Cooper et al., CID, 46:1310-1314, 2008; and Payette et al., Intervirology, 49:144-151, 2006). Although ODN-containing adjuvants appear to improve the immune response in some populations of hypo-responsive subjects (e.g., older healthy adults, and patients with end stage renal disease), during development of the present disclosure, HEPLISAV was found to remarkably improve the immune response to HBsAg in diabetic subjects. In particular, the present disclosure is based on the surprising finding that HEPLISAV induces a seroprotective anti-HBsAg response (defined as an anti-HBsAg level of > 10 mIU/ml) in diabetic subjects after only two doses. Specifically, HEPLISAV given as two doses over one month demonstrated a superior seroprotection rate as compared to a licensed HBV vaccine given as three doses over six months. This observation is in stark contrast to published recommendations for administration of four doses containing twice the concentration of HBsAg to hypo-responsive populations.

Hypo-responsive Subjects

Microbial infections cause an increase in morbidity and mortality in several patient populations. For instance, influenza virus infection is more likely to cause serious disease in the elderly, patients having pre-existing cardiovascular, renal, diabetic or pulmonary disease, and immunocompromised individuals (Dorrell et al., International Journal of STD & AIDS, 8:776-770, 1997). Individuals with diabetes mellitus have a higher incidence of infection than non-diabetic individuals. The increase in susceptibility to infection by diabetics is in large part a consequence of defects in their immune response (Geerlings and Hoepelman, FEMS Immunol Med Microbiol, 26:259-265, 1999).

Diabetic subjects have been reported to mount a suboptimal immune response following hepatitis B vaccination (Pozzilli et al., Diabetologia, 30:817-819, 1987; and Alavian and Tabatabaei, Vaccine, 28:3773-3777, 2010). For this reason, supplementary hepatitis B vaccinations are recommended for diabetic patients (Douvin et al., Diabetes Care, 20:148-151, 1997; and Wismans et al., J Med Virol, 35:216-222, 1991). Similarly, double doses of an HBV vaccine and/or a further booster are indicated for patients with renal disease (Beran, Expert Opin Biol Ther, 8:235-247, 2008; and Alavian and Tabatabaei, supra, 2010).
[0029] The present disclosure provides methods and compositions for inducing a seroprotective immune response to hepatitis B virus surface antigen in hypo-responsive subjects. In some embodiments, the hypo-responsive subject is an individual with a glucose metabolism disorder. In some embodiments, the glucose metabolism disorder is type I diabetes, type II diabetes, or pre-diabetes.

[0030] Diabetes mellitus is a heterogeneous group of metabolic diseases that lead to chronic elevation of glucose in the blood (hyperglycemia). Diabetes is characterized by pancreatic islet destruction or dysfunction leading to loss of glucose regulation. The two major types of diabetes mellitus are type I diabetes, also known as insulin-dependent diabetes (IDDM) or juvenile-onset diabetes, and type II diabetes, also known as non-insulin dependent (NIDDM) or adult-onset diabetes.

[0031] Type I diabetes results from an autoimmune-mediated destruction of pancreatic beta-cells. This results in a loss of insulin production and hyperglycemia. Type I diabetics require insulin replacement therapy to regulate their blood glucose levels.

[0032] Type II diabetes, in contrast, is characterized by hyperglycemia in the presence of higher-than-normal levels of plasma insulin (hyperinsulinemia). Type II diabetes represents over 90% of all cases, and occurs most often in overweight adults over 40 years of age. Progression of type II diabetes is associated with an increase in blood glucose, coupled with a relative decrease in the rate of glucose-induced insulin secretion. In type II diabetes, physiological processes that control carbohydrate metabolism are thought to have decreased sensitivity to insulin. Thus, treatment of type II diabetes frequently does not require administration of insulin, but may instead be based on diet and lifestyle changes, augmented by therapy with oral hypoglycemic agents.

[0033] Pre-diabetes is a condition in which blood glucose levels are higher than normal, yet below that for a diagnosis of diabetes. This condition is sometimes called impaired fasting glucose (IFG) or impaired glucose tolerance (IGT), depending on the test used to diagnose it. People with pre-diabetes are at increased risk of developing type II diabetes, formerly called adult-onset diabetes or noninsulin-dependent diabetes.

[0034] Injectable insulin replacement therapy utilizes one or more of rapid-acting, short-acting, intermediate-acting and long-acting insulin formulations (WebMD at “diabetes.webmd.com/diabetes-types-insulin”). Rapid-acting insulin formulations include but are not limited to Humalog, Lispro, Novolog, Aspart, Apidra and Glulisine. Short-acting insulin formulations include but are not limited to Humulin, Novolin and Velosulin. Intermediate-acting insulin formulations include but are not limited to NPH (N) and Lente (L). Long-acting insulin formulations include but are not limited to Ultralente (U), Lantus, Levemir and Detemir.
[0035] Oral hypoglycemic agents for treatment of type II diabetes include but are not limited to biguanides, sulfonylureas, meglinides, thiazolidinediones, and alpha-glucosidase inhibitors (U.S. Dept. Health Human Services at “www.ahrq.gov”). Metformin is a biguanide marketed as Glucophage. Sulfonylureas include but are not limited to tobutamide, acetohexamamide, tolazamide, chlorpropamide, glimepiride, glipizide, glyburide and gliclazide. Meglinides include but are not limited to repaglinide and nateglinide. Thiazolidinediones include but are not limited to pioglitazone and rosiglitazone. Alpha-glucosidase inhibitors include but are not limited to acarbose and miglitol.

**Immunostimulatory Sequences (ISS)**


[0037] Bacterial DNA and synthetic oligonucleotides containing ISS have multiple effects on the immune system. These include induction of B-cell proliferation and immunoglobulin production, secretion of interferon (IFN)-α, IFN-β, interleukin (IL)-12, and IL-18 by macrophages and IFN-γ secretion from natural killer cells (Krieg et al., Nature, 374:546-549, 1995; Klinman et al., Proc Natl Acad Sci USA, 93:2879-2883, 1996; and Messina et al., J Immunol, 147:1759-1764, 1991; Sato et al., Science, 273:352-354, 1996; Yamamoto et al., Jpn J Cancer Res, 79:866-873, 1988; Halpern et al., Cell Immunol, 167:72-78, 1996; Roman et al., Nat Med, 3:849-854, 1997; and Cowdery et al., J Immunol, 156:4570-4575, 1996). Therefore, ISS appears to stimulate the innate immune system to produce IFN-γ and inducers of IFN-γ (IFN-α, IFN-β, IL-12 and IL-18) and foster a cytokine milieu that greatly facilitates the induction of T cells that provide help for antibody production, especially those of the T-helper1 (Th1) phenotype.

[0038] Researchers at Dynavax (Berkeley, CA) have identified a 22-mer phosphorothioate 2′-deoxyribo nucleotide, 1018 ISS, that contains a specific sequence that can substantially enhance the immune response to co-administered antigens. 1018 ISS was chosen after screening a broad
panel of oligonucleotides for immunostimulatory activity in vitro and in vivo. 1018 ISS (5’-TGACTGTGAA CGTTCGAGAT GA-3’, set forth as SEQ ID NO:1) is active in mice, rabbits, dogs, baboons, cynomolgus monkeys and in vitro in human cells. Co-administration of 1018 ISS with protein antigens profoundly influences the magnitude and quality of the immune response to the antigens, including an increase in the overall antibody response to antigens. Consistent with this Th1-type response, 1018 ISS also significantly increases cytotoxic T lymphocyte (CTL) responses to protein antigens (Cho et al., Nat Biotechnol, 18:509-514, 2000; and Tighe et al., Eur J Immunol, 30:1939-1947, 2000).

[0039] The methods and compositions of the present disclosure comprise an ISS comprising an unmethylated cytosine-phosphate-guanosine (CpG) motif. In some preferred embodiments, the ISS comprises TCG in which the C is unmethylated, and which is from 8 to 100 nucleotides, preferably 8 to 50 nucleotides, or preferably 8 to 25 nucleotides in length. In some embodiments, the ISS is a 1018 ISS or a 1018 ISS-like oligonucleotide. The 1018 ISS consists of 22 nucleotides. The 1018-like ISS comprises 5’-AACGTTCG-3’. In some embodiments, the 1018-like ISS is at least 10, 15, or 20 nucleotides in length. In some preferred embodiments, the 1018 ISS-like oligonucleotide is less than 100 nucleotides in length, preferably less than 50, 40 or 30 nucleotides in length. For the sake of brevity, the CpG-containing ISS, the TCG-containing ISS, the 1018 ISS and the 1018 ISS-like oligonucleotides of the present disclosure are referred to below simply as an “ISS of the present disclosure” or “ISS.” In some embodiments, the ISS is single-stranded, while in other embodiments, it is double-stranded. In some preferred embodiments, the ISS comprises a phosphate backbone modification. In some preferred embodiments, the ISS comprises a phosphorothioate backbone modification. In some embodiments, the ISS comprises: 5’-TCGTCGTATT TTGTCGTTTTTTGTCGTATT-3’ (SEQ ID NO:2).

[0040] Specifically, an ISS of the present disclosure may contain modifications. Modifications include any known in the art, but are not limited to, modifications of the 3’OH or 5’OH group, modifications of the nucleotide base, modifications of the sugar component, and modifications of the phosphate group. Modified bases may be included in the palindromic sequence of the ISS as long as the modified base(s) maintains the same specificity for its natural complement through Watson-Crick base pairing (e.g., the palindromic portion is still self-complementary). The ISS may be linear, circular or include circular portions and/or a hairpin loop. The ISS may be single stranded or double stranded. The ISS may be DNA or RNA.

[0041] The ISS may contain naturally-occurring or modified, non-naturally occurring bases, and may contain modified sugar, phosphate, and/or termini. For example, in addition to phosphodiester linkages, phosphate modifications include, but are not limited to, methyl phosphonate, phosphorothioate, phosphoramidate (bridging or non-bridging), phosphotriester and
phosphorodithioate and may be used in any combination. Other non-phosphate linkages may also be used. In some embodiments, polynucleotides comprise only phosphorothioate linkages. In some embodiments, polynucleotides comprise only phosphodiester linkages. In some embodiments, an ISS may comprise a combination of phosphate linkages in the phosphate backbone such as a combination of phosphodiester and phosphorothioate linkages.

[0042] An ISS of the present disclosure can be synthesized using techniques and nucleic acid synthesis equipment, which are well known in the art including, but not limited to, enzymatic methods, chemical methods, and the degradation of larger oligonucleotide sequences. Naturally occurring DNA or RNA, containing phosphodiester linkages, is generally synthesized by sequentially coupling the appropriate nucleoside phosphoramidite to the 5'-hydroxy group of the growing oligonucleotide attached to a solid support at the 3'-end, followed by oxidation of the intermediate phosphate triester to a phosphate triester. Once the desired polynucleotide sequence has been synthesized, the polynucleotide is removed from the support, the phosphate triester groups are deprotected to phosphate diesters and the nucleoside bases are deprotected using aqueous ammonia or other bases (see, e.g., Beaucage, “Oligodeoxyribonucleotide Synthesis” in Protocols for Oligonucleotides and Analogs, Synthesis and Properties (Agrawal, ed.) Humana Press, Totowa, NJ, 1993).

[0043] The ISS can also contain phosphate-modified polynucleotides, some of which are known to stabilize the polynucleotide. Accordingly, some embodiments include stabilized immunomodulatory polynucleotides. Synthesis of polynucleotides containing modified phosphate linkages or non-phosphate linkages is also known in the art (see, e.g., Matteucci “Oligonucleotide Analogs: an Overview” in Oligonucleotides as Therapeutic Agents, (Chadwick and Cardew, ed. John Wiley and Sons, New York, NY, 1997). The phosphorous derivative (or modified phosphate group), which can be attached to the sugar or sugar analog moiety in the polynucleotides of the present disclosure can be a monophosphate, diphosphate, triphosphate, alkylphosphonate, phosphorothioate, phosphorodithioate, phosphoramidate or the like. The preparation of the above-noted phosphate analogs, and their incorporation into nucleotides, modified nucleotides and oligonucleotides is known in the art, and therefore not described here in detail (Peyrottes et al., Nucleic Acids Res. 24:1841-1848, 1996; Chaturvedi et al., Nucleic Acids Res. 24:2318-2323, 1996; and Schultz et al., Nucleic Acids Res. 24:2966-2973, 1996). For example, synthesis of phosphorothioate oligonucleotides is similar to that described above for naturally occurring oligonucleotides except that the oxidation step is replaced by a sulfurization step (Zon “Oligonucleoside Phosphorothioates” in Protocols for Oligonucleotides and Analogs, Synthesis and Properties (Agrawal, ed.) Humana Press, pp. 165-190, 1993). Similarly the synthesis of other phosphate analogs, such as phosphotriester (Miller et al., JACS 93:6657-6665,
1971), non-bridging phosphoramidates (Jager et al., *Biochem.* 27:7247-7246, 1988), N3' to P5' phosphoramidates (Nelson et al., *JOC* 62:7278-7287, 1997) and phosphorodithioates (U.S. Patent No. 5,453,496) has also been described. Other non-phosphorous based modified oligonucleotides can also be used (Stirchak et al., *Nucleic Acids Res.* 17:6129-6141, 1989). Polynucleotides with phosphorothioate backbones can be more immunogenic than those with phosphodiester backbones and appear to be more resistant to degradation after injection into the host (Braun et al., *J. Immunol.* 141:2084-2089, 1988; and Latimer et al., *Mol. Immunol.* 32:1057-1064, 1995).

**Hepatitis B Surface Antigen (HBsAg)**

Methods for preparing HBsAg are well documented (see, Valenzuela et al., *Nature* 298:347-350, 1982; U.S. Patent Nos. 4,710,463, 6,268,122, 6,270,955, and 6,297,355 to Murray; U.S. Patent Nos. 4,769,238, 6,475,489, and 6,544,757 to Rutter et al.). As used herein, the expression “hepatitis B surface antigen” or “HBsAg” includes any HBsAg antigen or fragment thereof displaying the antigenicity of the HBV surface antigen. In addition to the 226 amino acid sequence of the HBsAg S antigen (Tioillaus et al., *Nature*, 317:489, 1985), HBsAg may, if desired, contain all or part of a pre-S sequence. HBsAg as used herein may also refer to mutants, for example the “escape mutant” wherein HBsAg comprises a substitution of glycine to arginine at position 145. In preferred embodiments, the HBsAg is in particle form. In preferred embodiments, the HBsAg is a particle produced recombinantly in yeast. In other embodiments, the HBsAg is produced recombinantly in mammalian cells. In other embodiments, the HBsAg is purified from the plasma of an infected subject.

Four serotypes or subtypes of the hepatitis B surface antigen (HBsAg) have been defined by common determinant (a) and two mutually exclusive determinant pairs (d/y and w/r). These subtypes are adw, ayw, adr and ayr (Magnius and Norder, *Intervirology*, 38:24-34, 1995). The immunogenic composition of the present disclosure are suitable for immunizing a hyporesponsive subject against infection caused by all subtypes of HBV.

**Immunogenic Compositions and Administration Thereof**

The immunogenic compositions for use with the methods disclosed herein, comprise 1018 ISS or a 1018 ISS-like oligonucleotide and a hepatitis B virus surface antigen. The immunogenic compositions may further comprise an additional adjuvant and/or a pharmaceutically acceptable excipient. Pharmaceutically acceptable excipients, including buffers, are well known in the art (see, e.g., *Remington: The Science and Practice of Pharmacy*, 20th edition, Mack Publishing, 2000).

Upon administration, compositions comprising an antigen, 1018 ISS or a 1018 ISS-like oligonucleotide, and optionally an additional adjuvant lead to a potentiation of an immune response to the antigen. Adjuvants are known in the art and include, but are not limited to, oil-in-
water emulsions, water-in oil emulsions, alum (aluminum salts), liposomes and microparticles, including but not limited to, polystyrene, starch, polyphosphazene and polylactide/polyglycosides. Other suitable adjuvants also include, but are not limited to, MF59, DETOX™ (Ribi), squalene mixtures (SAF-1), muramyl peptide, saponin derivatives, mycobacterium cell wall preparations, monophosphoryl lipid A, mycolic acid derivatives, nonionic block copolymer surfactants, Quil A, cholera toxin B subunit, polyphosphazene and derivatives, and immunostimulating complexes (Takahashi et al., Nature 344:873-875, 1990), as well as others described herein. For veterinary use and for production of antibodies in non-human animals, mitogenic components of Freund's adjuvant (both complete and incomplete) can be used.

[0048] As with all immunogenic compositions, the immunologically effective amounts and method of administration of the particular formulation can vary based on the individual, what condition is to be treated and other factors evident to one skilled in the art. One factor to be considered includes the antigenicity of antigen, whether or not the ISS is administered in a mixture with, non-covalently associated with or covalently attached to the antigen. Other factors to be considered are the route of administration, number of doses to be administered, and time period between doses. A suitable dosage range is one that provides the desired modulation of immune response (e.g., stimulation of a seroprotective anti-HBsAg response).

[0049] In some embodiments, the immunogenic composition comprises from 1 μg to 50 μg HBsAg, preferably 4 to 40 μg HBsAg. In some preferred embodiments, the immunogenic composition comprises from 5 μg to 25 μg HBsAg (e.g., 5, 10, 15, 20 or 25 μg HBsAg), or more preferably from 10 μg to 20 μg HBsAg (e.g., 10, 15, or 20 μg HBsAg). In an exemplary embodiment, the immunogenic composition comprises 20 μg HBsAg. In some embodiments, the immunogenic composition comprises from 100 μg to 5000 μg of 1018 ISS or 1018 ISS-like oligonucleotide. In some preferred embodiments, the immunogenic composition comprises from 300 μg to 3000 μg, or more preferably from 500 μg to 5000 μg ISS (e.g., 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500 or 5000 μg ISS), or more preferably 1000 μg to 3000 μg ISS (e.g., 1000, 1500, 2000, 2500, 3000 μg ISS).

[0050] Routes of administration include but are not limited to topical, dermal, transdermal, transmucosal, epidermal, subcutaneous, parenteral, gastrointestinal, and naso-pharyngeal and pulmonary, including transbronchial and transalveolar. In a preferred embodiment, the immunogenic composition is administered by intradermal injection. In a preferred embodiment, the immunogenic composition is administered by intramuscular injection.

[0051] In some embodiments, the immunogenic compositions of the present disclosure comprise HBsAg and a further antigen. In some preferred embodiments, the further antigen is an
inactivated hepatitis A virus. In additional embodiments, the further antigen comprises one or more of the group consisting of an inactivated hepatitis A virus, a diptheria toxoid, a tetanus toxoid, acellular pertussis, *Haemophilus influenzae* Type B (HiB), an inactivated polio virus, and an influenza virus (FLU) Particular combination vaccines within the scope of the present disclosure include: Diptheria-Tetanus-Pertussis-Hepatitis B (DTP-HB), Diptheria-Tetanus-Hepatitis B (DT-HB), and Diptheria-Tetanus-Pertussis-Haemophilus-Hepatitis B (DTP-HiB-HiB). Additional combinations include: Influenza-Hepatitis B (FLU-HB), and Hepatitis A-Hepatitis B (HA-HB) Other combination vaccines of the present disclosure include Human Papilloma Virus (HPV) antigen-Hepatitis B (HPV-HB), and Varicella Zoster Virus (VZV)-Hepatitis B (VZV-HB).

In some embodiments, the present disclosure provides kits that comprising an immunogenic composition and a set of instructions relating to the use of the immunogenic composition for the methods describe herein. The kits may comprise an immunogenic composition packaged appropriately. For example, if the immunogenic composition is freeze-dried power, a vial with a resilient stopper is normally used so that the powder may be easily resuspended by injecting fluid through the resilient stopper. In some embodiments, the kits comprise a device for administration (e.g., syringe and needle). The instructions relating to the use of the immunogenic composition generally include information as to dosage, schedule and route of administration for the intended methods of use.

**EXAMPLES**

**Abbreviations:** GMC (geometric mean concentration); HBcAb or anti-HBc (hepatitis B core antibody); HBcAg (hepatitis B core antigen); antibody to HBsAg or anti-HBsAg (hepatitis B surface antibody); HBsAg (hepatitis B surface antigen); HBV (hepatitis B virus); HEPLISAV (recombinant HBsAg+1018 ISS vaccine of Dynavax); ISS (immunostimulatory sequence); ITT (intent-to-treat); PP (per-protocol); mIU/mL (milli international units/milliliter); and SPR (seroprotective immune response, defined as [anti-HBsAg] of ≥10 mIU/mL).

**EXAMPLE 1**

**Immunogenicity of HEPLISAV Compared to an Approved Recombinant HBsAg Vaccine**

This example provides a description of a multicenter, phase three clinical study conducted among healthy adults, which compared two doses of HEPLISAV (HBsAg + 1018 ISS vaccine of Dynavax, Berkeley, CA) to three doses of ENGERIX-B (HBsAg adsorbed to alum vaccine of GlaxoSmithKline, Research Triangle Park, NC). An ad-hoc analysis was conducted to compare the immunogenicity of these two vaccine regimens among persons with type II diabetes. Diabetes was assessed by the review of recorded subject medical history and prior/concomitant use of oral hypoglycemics and/or insulin.
Primary Immunogenicity Objective: To compare the proportion of subjects who exhibit seroprotective immune response (SPR = anti-HBsAg antibody level ([anti-HBsAg]) = 10 mIU/mL) when measured at Week 12 following vaccination with HEPLISAV at months 0 and 1 to the proportion of subjects who exhibit SPRs when measured at Week 28 following vaccination with ENGERIX-B at months 0, 1 and 6.

Secondary Immunogenicity Objective(s): To compare the proportion of subjects exhibiting an SPR for HEPLISAV versus ENGERIX-B when measured at Week 4.

Exploratory Immunogenicity Objective(s): To compare the proportion of subjects exhibiting an SPR for HEPLISAV versus ENGERIX-B when measured at Weeks 8, 12, 24 and 28. To describe the anti-HBsAg serum geometric mean concentrations (GMCS) observed for HEPLISAV and ENGERIX-B when calculated at Weeks 4, 8, 12, 24 and 28 (durability of response). To compare the proportion of subjects who exhibit seroprotective immune response (SPR = [anti-HBsAg] = 10 mIU/mL) when measured at Week 8 following vaccination with HEPLISAV at Weeks 0 and 4 to the proportion of subjects who exhibit SPR when measured at Week 28 following vaccination with ENGERIX-B at Weeks 0, 4, and 24.

Study Design. This study was conducted as a subject- and observer-blinded, randomized, controlled study of adult subjects (ages 11 to 55 years) randomized 3:1 to receive injections with either HEPLISAV (3000 µg 1018 ISS + 20 µg rHBsAg) or ENGERIX-B (20 µg rHBsAg in alum). Subjects were stratified by age (11 to 39 years of age versus 40 to 55 years of age) prior to randomization. All subjects received a total of three intramuscular injections (active vaccine or matching placebo), given on study Day 0, Week 4 (1 month), and Week 24 (6 month). Subjects randomized to ENGERIX-B received three injections of ENGERIX-B (20 µg rHBsAg adsorbed to alum) at Weeks 0, 4 and 24. Subjects randomized to HEPLISAV received an injection of 3000 µg 1018 ISS + 20 µg rHBsAg at Weeks 0 and 4, and a saline placebo at Week 24. All subjects were asked to return approximately 4 weeks after each injection to have blood drawn to measure anti-HBsAg levels and to undergo safety evaluations.

Study Population. Subjects were selected from among HBV seronegative male and female volunteers. Inclusion and exclusion criteria met by study participants included but were not limited to the listing provided below. Inclusion Criteria: 11 to 55 years of age; and serum negative for HBsAg, anti-HBsAg and anti-HBcAg.Exclusion Criteria: history of HBV infection; prior immunization with any HBV vaccine; clinically debilitating illness, (e.g., fever = 38°C within 72 hours prior to study injection, bleeding disorders, cancer, autoimmune disease, immunodeficiency, etc.); history or laboratory evidence of autoimmune disease; high risk for recent exposure to HBV, HCV or HIV; recent receipt of blood products or likely to require infusion of blood products; previously received DNA plasmids or oligonucleotides by injection;
and recent use of systemic corticosteroids, other immunomodulators or other immunosuppressive medications (with the exception of inhaled steroids); and history of sensitivity to any component of the study vaccines.

[0060] Subjects included in the diabetes cohort were selected based on a medical history including preferred terms with DIABET, and excluding GESTATIONAL DIABETES. Additional subjects in the diabetes cohort were selected based on the use of concomitant medication (ATC code A10) for diabetes, in the absence of a documented medical history of diabetes.

[0061] **Treatments Administered.** HEPLISAV (3000 µg 1018 ISS + 20 µg rHBsAg) was manufactured by Rentschler Biotechnologie GmbH, Laupheim, Germany for Dynavax Technologies Corporation, Berkeley, CA. The rHBsAg of this formulation was derived from yeast cells transformed with an expression vector containing HBsAg (S) sequence, subtype adw. 1018 ISS is a single-stranded, 22-base phosphorothioate 2′-deoxyribo-oligonucleotide prepared by standard solid-phase chemistry techniques (5′-TGACTGTGAA CGTTCGAGAGA-3′, set forth as SEQ ID NO:1). 1018 ISS has a molecular mass of approximately 7150 Daltons. HEPLISAV also contains the following excipients: 8 mM sodium phosphate, 154 mM sodium chloride, and 0.01% w/w polysorbate 80, pH 7.0 buffer. The HEPLISAV drug product is formulated as 6000 mcg/mL 1018 ISS and 40 mcg/mL HBsAg in a 2-mL vial containing 0.7 mL of solution (28 mcg of protein and 4200 mcg of 1018 ISS per vial) of which a 0.5 mL dose (20 mcg of protein and 3000 mcg of 1018 ISS) is administered. HEPLISAV is stored at 2 to 8 °C before use.

[0062] **Immunogenicity Analyses.** Two patient populations were considered for the immunogenicity analysis: the per-protocol (PP) population and the intent-to-treat (ITT) population. The immunogenicity analysis using the PP population was considered primary. **PP Population – Immunogenicity:** The PP population included subjects who met the eligibility criteria, did not violate the protocol in a substantial manner, received all protocol-specified study vaccinations, had their primary serology and all vaccinations within the specified day ranges, and had serology at their primary endpoint (week 12 for HEPLISAV and week 28 for ENGERIX-B). **ITT Population – Immunogenicity:** The ITT population included subjects who had at least one vaccination and one post baseline anti-HBsAg level. Anti-HBsAg was measured by using the hepatitis B enhanced chemiluminescence immunoassay (Hep B ECI, Ortho Clinical Diagnostics, Rochester, NY).

[0063] All statistical tests comparing demographic, patient characteristic and safety data were two-sided and conducted at the 5% significance level. All immunogenicity analyses utilized one-
sided tests at the 2.5% level of significance. All data analyses were performed using Statistical Analysis Systems (SAS) for Windows 95/NT (version 8.2 or later, SAS Institute, Cary, NC).

[0064]  *Seroprotective Immune Response (SPR) Rate.* For the purpose of this analysis, a seroprotective immune response was defined as an anti-HBsAg concentration of = 10 mIU/mL.

[0065]  *Geometric Mean Concentrations (GMCs).* Anti-HBsAg GMC was measured four weeks after each active injection for both groups. All anti-HBsAg concentrations that were reported as <5.0 mIU/mL were considered as 2.5 mIU/mL in the computation for GMC. Log (base 10)-transformed anti-HBsAg concentrations were used to summarize the GMCs for the two treatment groups.

*Results.* Of the 2101 non-diabetic and diabetic subjects in the overall per protocol study population (1566 HEPLISAV, and 535 ENGERIX-B), the SPR was 95% at Week 12 in the HEPLISAV group and 81% at Week 28 in the ENGERIX-B group (p<0.001), indicating non-inferiority/superiority of HEPLISAV. Among the 62 diabetics in the per protocol population, 45 were in the HEPLISAV group (mean age of 44.4 years) and 17 in the ENGERIX-B group (mean age of 45.5 years). Of these subjects, 38 (84%) in the HEPLISAV group achieved SPR compared to 0 (0%) in the ENGERIX-B group at Week 12 (p<0.0001), and 42 (93%) versus 6 (35%) respectively at Week 28 (p<0.0001).

**Table 1-1. Seroprotection (SPR) Rates of HBsAg Vaccinated Study Populations**

<table>
<thead>
<tr>
<th>Week / Population</th>
<th>HEPLISAV # Subjects</th>
<th>HEPLISAV SPR Rate</th>
<th>ENGERIX-B # Subjects</th>
<th>ENGERIX-B SPR Rate</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Week 12</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP non-diabetic</td>
<td>1,521</td>
<td>95.4%</td>
<td>518</td>
<td>23.2%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PP diabetic</td>
<td>45</td>
<td>84.4%</td>
<td>17</td>
<td>0.0%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ITT diabetic</td>
<td>54</td>
<td>85.2%</td>
<td>18</td>
<td>0.0%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Week 28</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP non-diabetic</td>
<td>1,520</td>
<td>98.1%</td>
<td>518</td>
<td>82.6%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PP diabetic</td>
<td>45</td>
<td>93.3%</td>
<td>17</td>
<td>35.3%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ITT diabetic</td>
<td>54</td>
<td>92.6%</td>
<td>18</td>
<td>33.3%</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Table 1-2. Seroprotection (SPR) Rates in Vaccinated Per Protocol Diabetic Subjects

<table>
<thead>
<tr>
<th>Week / Population</th>
<th>HEPLISAV # Subjects</th>
<th>HEPLISAV SPR Rate</th>
<th>ENGERIX-B # Subjects</th>
<th>ENGERIX-B SPR Rate</th>
<th>P-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Week 4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP diabetic</td>
<td>45</td>
<td>24.4%</td>
<td>17</td>
<td>0%</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Week 8</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP diabetic</td>
<td>45</td>
<td>71.1%</td>
<td>17</td>
<td>5.9%</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Week 12</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP diabetic</td>
<td>45</td>
<td>84.4%</td>
<td>17</td>
<td>0%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Week 24</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP diabetic</td>
<td>45</td>
<td>93.3%</td>
<td>17</td>
<td>11.8%</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Week 28</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP diabetic</td>
<td>45</td>
<td>93.3%</td>
<td>17</td>
<td>35.3%</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* ND = Not Done.

Table 1-3. Anti-HBsAg Geometric Mean Concentrations (GMC) in Vaccinated Per Protocol Diabetic Subjects+

<table>
<thead>
<tr>
<th>Week / Population</th>
<th>HEPLISAV # Subjects</th>
<th>HEPLISAV GMC</th>
<th>ENGERIX-B # Subjects</th>
<th>ENGERIX-B GMC</th>
<th>P-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Week 4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP diabetic</td>
<td>45</td>
<td>4.8 (3.5, 6.6)</td>
<td>17</td>
<td>2.5 (2.5, 2.5)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Week 8</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP diabetic</td>
<td>45</td>
<td>36.7 (21.3, 63.1)</td>
<td>17</td>
<td>3.2 (2.2, 4.6)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Week 12</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP diabetic</td>
<td>45</td>
<td>47.1 (29.4, 75.3)</td>
<td>17</td>
<td>2.8 (2.4, 3.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Week 24</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP diabetic</td>
<td>45</td>
<td>109.8 (68.2, 176.7)</td>
<td>17</td>
<td>3.2 (2.2, 4.7)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Week 28</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP diabetic</td>
<td>45</td>
<td>96.9 (59.4, 158.3)</td>
<td>17</td>
<td>16.7 (3.8, 74.0)</td>
<td>0.0283</td>
</tr>
</tbody>
</table>

+ GMC (95% confidence interval).

* ND = Not done.

[0066] As determined during development of the present disclosure, in a subset analysis of adults with diabetes, HEPLISAV given as two doses over one month demonstrated superior SPR compared to ENGERIX-B given as three doses over six months. Thus, use of HEPLISAV to vaccinate diabetics provides superior protection against hepatitis B infection.
and disease as compared to a Food and Drug Administration approved recombinant HBV vaccine.

[0067] Although the foregoing disclosure has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent to those skilled in the art that certain changes and modifications may be practiced. Therefore, descriptions and examples should not be construed as limiting the scope of the disclosure.
CLAIMS

We claim:

1. A method for eliciting an immune response against hepatitis B virus (HBV) in a human subject having a glucose metabolism disorder, comprising:

   administering to a human subject a first and a second dose of an immunogenic composition on at least two separate occasions, wherein said immunogenic composition comprises a hepatitis B surface antigen (HBsAg), and an immunostimulatory sequence (ISS) of from 8 to 50 nucleotides in length comprising an unmethylated cytosine-phosphate-guanosine (CpG) motif,

   wherein said subject has a glucose metabolism disorder selected from the group consisting of type I diabetes, type II diabetes, and pre-diabetes at the onset of said administering; and

   wherein said HBsAg and said ISS are present in said immunogenic composition in amounts effective to elicit an immune response in said subject at least two months after said second dose.

2. The method of claim 1, wherein the ISS comprises the nucleotide sequence 5’-TCG-3’.

3. The method of claim 1, wherein the ISS comprises the nucleotide sequence ‘5-CGTTTCG-3’ or ‘5-AACGTTTCG-3’.

4. The method of claim 1, wherein said ISS comprises the nucleotide sequence of SEQ ID NO:1.

5. The method of claim 1, wherein said ISS comprises a phosphate backbone modification.

6. The method of claim 1, wherein said ISS comprises a phosphorothioate backbone modification.

7. The method of claim 1, wherein said ISS is 1018 ISS.
8. The method of claim 1, wherein said immunogenic composition comprises 20 μg or less of said HBsAg.

9. The method of claim 1, wherein said immunogenic composition comprises 3000 μg or less of said ISS.

10. The method of claim 1, wherein said HBsAg is a recombinant HBsAg produced in yeast.

11. The method of claim 1, wherein said immune response is a seroprotective response comprising an anti-HBsAg response of at least 10 mIU/mL at least two months after said second dose.

12. The method of claim 11, wherein said anti-HBsAg response is at least 15, 20, or 25 mIU/mL at least two months after said second dose.

13. The method of claim 1, wherein said immune response is a seroprotective response comprising an anti-HBsAg response of at least 10 mIU/mL at least six months after said second dose.

14. The method of claim 13, wherein said anti-HBsAg response is at least 20, 30, 40 or 50 mIU/mL at least six months after said second dose.

15. The method of claim 1, wherein the subject has type II diabetes.

16. The method of claim 15, wherein the subject is taking one or both of an oral hypoglycemic and insulin, at the onset of said administering.

17. The method of claim 16, wherein the oral hypoglycemic comprises one or more of the group consisting of a biguanide, a sulfonylurea, a nonsulfonylurea secretagogue, an alpha glucosidase inhibitor, and a thiazolidinedione.

18. The method of claim 16, wherein the insulin is recombinant human insulin or an analog thereof.
19. The method of claim 1, wherein the subject has a body mass index of greater than 25 (overweight).

20. The method of claim 1, wherein the subject is a resident of a nursing home or an assisted living facility.

21. The method of claim 1, wherein the subject is over 40 years of age.

22. The method of claim 1, wherein the HBsAg comprises the S antigen.

23. The method of claim 22, wherein the HBsAg further comprises the pre-S2 antigen.

24. The method of claim 23, wherein the HBsAg further comprises the pre-S1 antigen.

25. A method for eliciting an immune response against hepatitis B virus (HBV) in a human subject having a glucose metabolism disorder, comprising:
   administering to a human subject an effective amount of an immunogenic composition, wherein said immunogenic composition comprises a hepatitis B surface antigen (HBsAg), and an immunostimulatory sequence (ISS) of from 8 to 50 nucleotides in length comprising an unmethylated cytosine-phosphate-guanosine (CpG) motif, and wherein said subject has a glucose metabolism disorder selected from the group consisting of type I diabetes, type II diabetes, and pre-diabetes at the onset of said administering.

26. A kit for eliciting an immune response against hepatitis B virus (HBV) in a human subject having a glucose metabolism disorder, comprising:
   an immunogenic composition comprising a hepatitis B surface antigen (HBsAg) and an immunostimulatory sequence (ISS) of from 8 to 50 nucleotides in length comprising an unmethylated cytosine-phosphate-guanosine (CpG) motif; and
   instructions for administering to a human subject an effective amount of said immunogenic composition, wherein, wherein said subject has a glucose metabolism disorder selected from the group consisting of type I diabetes, type II diabetes, and pre-diabetes.
27. An immunogenic composition comprising a 1018 immunostimulatory sequence (ISS) and a hepatitis B surface antigen (HBsAg) for use in eliciting an immune response against hepatitis B virus (HBV) in a human subject when administered as a first dose and a second dose on at least two separate occasions,

wherein the 1018 ISS and the HBsAg are present in said immunogenic composition in amounts effective to elicit an immune response in said subject at least two months after said second dose, and

wherein said subject has a glucose metabolism disorder selected from the group consisting of type I diabetes, type II diabetes, and pre-diabetes at the onset of said administering.

28. An immunogenic composition comprising a 1018 immunostimulatory sequence (ISS) and a hepatitis B surface antigen (HBsAg) for use in preventing a human subject from being infected with a hepatitis B virus (HBV) when administered as a first dose and a second dose on at least two separate occasions,

wherein the 1018 ISS and the HBsAg are present in said immunogenic composition in amounts effective to prevent said subject from becoming infected with HBV at least two months after said second dose, and

wherein said subject has a glucose metabolism disorder selected from the group consisting of type I diabetes, type II diabetes, and pre-diabetes at the onset of said administering.

29. An immunogenic composition comprising a 1018 immunostimulatory sequence (ISS) and a hepatitis B surface antigen (HBsAg) for use in preparing a medicament to elicit an immune response against hepatitis B virus (HBV) in a human subject when administered as a first dose and a second dose on at least two separate occasions,

wherein the 1018 ISS and the HBsAg are present in said immunogenic composition in amounts effective to elicit an immune response in said subject at least two months after said second dose, and

wherein said subject has a glucose metabolism disorder selected from the group consisting of type I diabetes, type II diabetes, and pre-diabetes at the onset of said administering.
30. An immunogenic composition comprising a 1018 immunostimulatory sequence (ISS) and a hepatitis B surface antigen (HBsAg) for use in preparing a medicament for preventing a human subject from being infected with a hepatitis B virus (HBV) when administered as a first dose and a second dose on at least two separate occasions,

wherein the 1018ISS and the HBsAg are present in said immunogenic composition in amounts effective to prevent said subject from becoming infected with HBV at least two months after said second dose, and

wherein said subject has a glucose metabolism disorder selected from the group consisting of type I diabetes, type II diabetes, and pre-diabetes at the onset of said administering.
Seroconversion (SPR) in Diabetics
(N = 45 for HEPLISAV; 17 for Engerix-B)

First injection:
- HEPLISAV: 93%
- Engerix-B: 84% (p = 0.0001)
- Placebo: 0%

Second injection:
- HEPLISAV: 35% (p = 0.0001)
- Engerix-B: 0%
- Placebo: 0%

Figure 1: % Seroconversion (Anti-HBs > 10 mIU/ml)
# INTERNATIONAL SEARCH REPORT

### A. CLASSIFICATION OF SUBJECT MATTER
- IPC(8) - A61K 48/00; C07H 21/02 (2011.01)
- USPC - 514/44R; 536/23.1

According to International Patent Classification (IPC) or to both national classification and IPC

### B. FIELDS SEARCHED

- Minimum documentation searched (classification system followed by classification symbols)
  - IPC(8) - A61K 48/00; C07H 21/02 (2011.01)
  - USPC - 514/44R; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
- PubWEST (PGPB, USPT, USOC, EPAB, JPAB); Google Scholar; PubMed: HBV, HBsAg, ISS, immunostimulatory sequence, diabetes, type II, seropositivity, 1018 ISS, phosphate, backbone, vaccine, insulin, hypoglycemia, S, pre-S1, pre-S2, oral, orally, biguanide, sulfonylurea, thiazolidinedione, body mass index, BMI, GenCore 6.3; SEQ ID NO:1

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>HALPERIN, et al. A Phase I Study of the Safety and Immunogenicity of Recombinant Hepatitis B Surface Antigen Co-Administered with an Immunostimulatory Phosphorothioate Oligonucleotide Adjuvant. Vaccine 2003, 21:2461-2467; fig 1: abstract; pg 2461, col 2, para 1-2; pg 2462, col 1, para 3; col 2, para 2 to pg 2463, col 1, para 1; pg 2466, col 1, para 2, col 2, para 2</td>
<td>1-30</td>
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  - "A" document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search: 16 July 2011 (16.07.2011)

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