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A phase I study of the safety and immunogenicity of recombinant hepatitis B surface antigen co-administered with an immunostimulatory phosphorothioate oligonucleotide adjuvant☆

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Abstract

Certain oligodeoxynuclotides with CpG motifs provide enhanced immune response to co-delivered antigens. We performed a phase I, observer-blinded, randomized study in healthy anti-hepatitis B surface antigen (anti-HBsAg) antibody negative adults to explore safety and immunogenicity of co-injection of recombinant HBsAg combined with an immunostimulatory DNA sequence (ISS) 1018 ISS. Four ISS dosage groups ($N = 12$ per group) were used: 300, 650, 1000 or 3000 μg. For each group, two controls received 20 μg HBsAg alone, two controls received ISS alone, and eight subjects received ISS + 20 μg HBsAg. Subjects received two doses 8 weeks apart. Injection site reactions (tenderness and pain on limb movement) were more frequent at higher ISS + HBsAg doses but were mainly mild and of short duration. Higher anti-HBsAg antibody levels were associated with higher ISS doses. Four weeks after the first dose, a seroprotective titer (≥10 mIU/ml) was noted for 0, 25, 75, and 87.5% of subjects by increasing ISS dose group ($P < 0.05$) for those who received ISS+HBsAg; 1 month after the second dose this increased to 62.5, 100, 100, and 100%, respectively. Geometric mean anti-HBsAg antibody levels by increasing ISS + HBsAg dose were 1.22, 5.78, 24.75, and 206.5 mIU/ml after the first dose and 65.37, 877.6, 1545, and 3045 mIU/ml after the second dose. We conclude that 1018 ISS + HBsAg was well tolerated and immunogenic in this phase I study in healthy adults and may offer the potential for enhancement of hepatitis B virus (HBV) immunization and protection after one or two doses or in individuals who fail to respond to the standard vaccine regimen.

Keywords: Hepatitis B vaccine; Immunostimulatory sequence; CpG motifs; Randomized controlled trial

1. Introduction

Hepatitis B virus (HBV) causes acute and chronic infection in humans and is responsible for substantial morbidity and mortality [1,2]. Although only 5–15% of acutely infected young children and 33–50% of older children and adults will manifest clinical illness, 5–10% of adults and as high as 90% of vertically infected infants will become chronically infected [3]. Chronically HBV infected individuals are at increased risk of subsequent development of hepatocellular carcinoma [4]. Worldwide, it is estimated that 5% of the world's population has chronic HBV infection and that 500,000 to 1 million people die annually from HBV-related liver disease [5]. Universal immunization against HBV is recommended throughout the world and is effective in preventing maternal to infant transmission and chronic infection, and in decreasing the incidence of hepatocellular carcinoma [6].

Currently available HBV vaccines in North America and Europe consist of recombinant hepatitis B surface antigen (HBsAg) adsorbed to aluminum hydroxide or aluminum phosphate. Protection against disease is associated with post-immunization antibody levels against HBsAg of ≥10 mIU/ml [7]. Protective levels are achieved in 90% or

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more healthy adults after a three-dose series of vaccine given at 0, 1, and 6 months although rates of seroprotection are lower with increasing age, obesity, and in those who smoke [8]. Although accelerated 3-month schedules (0, 1, and 2 months) are routinely used in some jurisdictions, higher antibody levels are achieved with longer intervals between the second and third injection [9]. Two-dose regimens with an interval of at least 4 months between doses have been evaluated for use in adolescents in whom the need for multiple doses may lead to decreased compliance with completion of the immunization series [10]. Similarly, in the developing world where hepatitis B remains a significant cause of morbidity and mortality, a single-dose regimen or an accelerated two-dose regimen would be useful to increase rates of HBV immunity [11]. A more immunogenic vaccine is also needed which would be effective in high-risk individuals who fail to respond to the standard immunization series.

Immunostimulatory DNA sequences (ISS) are emerging as useful tools for modulating immune responses. ISS are components of bacterial but not vertebrate DNA that have potent NK activation and interferon-inducing properties [12] which can be reproduced by certain synthetic oligonucleotides containing CpG motifs [13,14]. ISS stimulate the production of Th1-type cytokines such as IL-12 and interferons from a variety of cells such as dendritic cells, macrophages and NK cells [15–17]. ISS also stimulate B-cell proliferation and immunoglobulin secretion [18–20] as well as activation of antigen presenting cells [21,22]. ISS have potent Th1 adjuvant properties when used for immunization with DNA [23,24] or protein [17,25–27] vaccines. We studied the safety and immunogenicity of a 22-mer synthetic, phosphorothioate oligodeoxyribonuclotide immunostimulatory sequence (1018 ISS) co-administered with HBsAg in healthy adults.

2. Materials and methods

2.1. Vaccine

Study products consisted of 1018 ISS (sequence 5′-TGACTGTGAACGTTCGAGATGA-3′; Dynavax Technologies, Berkeley, CA) alone and in combination with yeast recombinant HBsAg without aluminum hydroxide adjuvant (Rhein Americana S.A., Buenos Aires, Argentina). Study products were stored at −60 °C or below and used within 8 h of thawing, and were diluted with sterile phosphate buffered saline to achieve the desired concentrations. Study vaccines were reconstituted by the study pharmacist to contain 300, 650, 1000, or 3000 μg of 1018 ISS alone or mixed with 20 μg HBsAg.

2.2. Populations

Healthy adults between 18 and 55 years of age were eligible if they had no history of hepatitis B infection or immunization with hepatitis B vaccine, had negative tests for HBsAg and antibodies against HBsAg and HBcAg. Individuals were excluded from enrollment if they were pregnant or unwilling to use effective contraception during the study, had clinically significant acute or chronic diseases, had any immunosuppressive disorders or medication, had prior injection of DNA plasmids or oligonuclotides, had behavioral risk factors that might have resulted in recent exposure to hepatitis B virus, had received blood products or immunoglobulin within 3 months of study entry, had a history of sensitivity to any component of the study vaccines or had abnormalities of screening blood chemistries, hematology, or urinalysis.

2.3. Study design and procedures

The study was designed as a single center, randomized, controlled, observer-blinded, dose escalating study in 48 healthy adult volunteers. Written, informed consent was obtained from all participants prior to any study procedure; the study was approved by the Research Ethics Board of the IWK Health Centre, Halifax, NS, Canada. The first 12 participants were randomly allocated in a ratio of 2:2:8 by computer generated list to receive either 20 μg HBsAg, 300 μg 1018 ISS, or 20 μg HBsAg + 300 μg 1018 ISS. Only the study pharmacist who mixed the vaccines at the time of immunization and who was not involved in any other aspects of the study was aware of the vaccine allocation. The second cohort of 12 participants was randomly allocated to receive 20 μg HBsAg, 650 μg ISS, or 20 μg HBsAg + 650 μg 1018 ISS in a ratio of 2:2:8. Dose escalation occurred after review by the investigator, sponsor, and an independent medical monitor of the safety results of the cohort who had received the previous dose level. The third and fourth cohorts of 12 subjects received 1000 or 3000 μg 1018 ISS with or without 20 μg HBsAg, respectively, in the same ratio.

Two doses of the same vaccine were given as intramuscular injections into opposite deltoid muscles 2 months apart. Participants were monitored by study personnel for 30 min after the immunization for any immediate adverse events and by the subjects themselves using a symptom diary for 7 days post-immunization. Participants were instructed to measure their temperature daily and to assess specific injection site adverse events (redness, swelling, warmth, tenderness, pain with arm movement) and systemic adverse events (chills, headache, muscle aches, fatigue, nausea, vomiting, diarrhea, joint pain); all reports of other adverse events were collected and categorized by body system. Serious adverse events were defined as events that were fatal or life-threatening; caused or prolonged hospitalization; resulted in a significant, persistent, or permanent disability; produced a congenital anomaly; or required intervention to prevent permanent impairment or damage. Solicited adverse events were either measured (fever, redness, swelling) or categorized as mild (awareness of symptom but easily tolerated),

moderate (discomfort enough to cause interference with usual activity), or severe (incapacitating with inability to work or do usual activity). Adverse events were collected by study personnel during visits 7 and 28 days after each immunization.

Blood was collected by venipuncture immediately before 1 week and 1 month after each dose of vaccine for measurement of serum biochemical and hematological parameters, complement (C3 and C4), erythrocyte sedimentation rate, and antinuclear and anti-single stranded and -double stranded DNA. Serum antibody response to HBsAg was measured by enzyme immunoassay (AUSAB EIA, Abbott Laboratories, Abbott Park); all testing was performed in a blinded fashion on code labeled, matched pre- and post-immunization sera. All anti-HBsAg antibody levels were expressed as mIU/ml. At the completion of the study, licensed hepatitis B vaccine was provided to participants who had not achieved antibody levels ≥100 mIU/ml.

2.4. Data analysis and statistical considerations

Adverse events were tabulated by time (day) and by severity (mild, moderate, and severe). The maximum size and severity was used within each time period. Clinically significant reactions were defined as measured reactions ≥10 mm, fever ≥38 °C, and severity ≥ moderate for other symptoms. Severe reactions were defined as measured reactions ≥50 mm, fever ≥39 °C, and severity "severe" for all other symptoms. Injection site reactions were combined to give an "any local" reaction category and all other reactions combined to give an "any general" reaction. The proportion of subjects having an adverse reaction was estimated by vaccine group, observation period and severity. Binomial distribution point estimates and 95% confidence intervals were used to estimate each rate; percentages were compared by Fisher's exact test. $P < 0.05$ was considered statistically significant; no adjustments were made for multiple comparisons.

Geometric mean antibody levels and 95% confidence intervals were estimated pre- and post-immunization and compared across groups by ANOVA; linear regression was used to assess trend across dose level. The proportion of each vaccine group achieving seroprotective antibody levels (≥10 mIU/ml) post-immunization was compared by Fisher's exact test.

The primary outcome of the study was the proportion of subjects reporting specific post-injection reactions. The secondary outcome was the proportion of subjects seroprotected after immunization and the geometric mean antibody level. As this was a phase I, first-in-human clinical trial, no formal hypothesis testing was planned and no formal sample size calculation was performed; however, each treatment group sample size (eight participants) ensured that the probability of detecting at least one adverse event in the group was 0.73, provided that the true adverse event rate exceeded 15%.

3. Results

3.1. Demographics

A total of 74 subjects provided written, informed consent and underwent pre-study screening; reasons for "screen failures" included persistently abnormal baseline biochemistry or hematology tests [14], pre-existing antibody against hepatitis B virus [4], hypertension [1], inability to contact after the screening visit [2], withdrawal of consent [1], target enrollment reached before subject could be scheduled [4]. The remaining 48 participants were randomized and received study drug; all but one completed the study. The mean age of participants was 33 years (range 18–52 years) and 63% were women. There were no differences in the age or gender distribution amongst the vaccine groups. All but one participant completed participation in the study; one subject withdrew consent because of adverse events after the first injection.

3.2. Adverse events

3.2.1. Clinical adverse events

No adverse events were reported in the first 30 min after injection. During the first 7 days after each dose, solicited adverse events were common; an adverse event was reported after the first dose by 2 (25%) HBsAg recipients, 6 (75%) of the combined ISS recipients, and 5 (62.5%) of the 300 μg ISS+HBsAg recipients, and 7 (87.5%) of each of the 650 μg, 1000 μg, and 3000 μg ISS+HBsAg recipients ($P = 0.04$ for the comparison of the latter 3 with HBsAg). After the second dose, solicited adverse events of any type were reported by 5 (62.5%), 5 (62.5%), 3 (37.5%), 1 (12.5%), 5 (71.4%) and 5 (62.5%) (P not significant for all comparisons). Both local and systemic adverse events were reported (Table 1). Injection site adverse events were for the most part reported within the first 24 h after the injection whereas the more non-specific systemic adverse events (such as headache, diarrhea) were reported throughout the first 7 days after the injection (data not shown). Mild tenderness at the injection site and mild pain on motion of the injected limb were the most commonly reported adverse events and were significantly more frequent in the 1000 μg and 3000 μg ISS + HBsAg groups for tenderness and the 3000 μg + HBsAg group for motion pain; these events were short and self limited and did not require any medical treatment or intervention. There was a significant ISS dose-related trend for increased tenderness ($P = 0.01$) and pain on limb motion ($P = 0.05$). Warmth at the injection site was only reported by three participants (one each in the HBsAg alone, 300 μg ISS+HBsAg and 1000 μg ISS + HBsAg groups). Only one severe (≥50 mm) injection site adverse event (redness) was reported; redness of 50 mm was reported after the first dose of 1000 μg ISS + HBsAg and resolved with 24 h without treatment. Injection site adverse events did not increase with the second dose compared to the first.

Table 1
Adverse events reported within 7 days after each of two doses of HBsAg and/or 1018 ISS and/or HBsAg combined with varying quantities of 1018 ISS in 48 healthy adult volunteers

Adverse event	Dose	Severity	Vaccine number (%) reporting adverse event					
			ISS[a] ($N = 8$)	HBsAg ($N = 8$)	HBsAg + 300 μg ISS ($N = 8$)	HBsAg + 650 μg ISS ($N = 8$)	HBsAg + 1000 μg ISS ($N = 8$)[b]	HBsAg + 3000 μg ISS ($N = 8$)
Redness	1	Any	0	0	0	0	1 (12.5)	1 (12.5)
		Moderate/severe	0	0	0	0	1 (12.5)	1 (12.5)
	2	Any	0	1 (12.5)	1 (12.5)	0	2 (28.6)	0
		Moderate/severe	0	1 (12.5)	1 (12.5)	0	1 (14.3)	0
Swelling	1	Any	0	0	1 (12.5)	1 (12.5)	1 (12.5)	0
		Moderate/severe	0	0	1 (12.5)	1 (12.5)	1 (12.5)	0
	2	Any	0	1 (12.5)	0	0	1 (14.3)	0
		Moderate/severe	0	0	0	0	0	0
Tenderness	1	Any	3 (37.5)	0	2 (25)	1 (12.5)	6 (75)[c]	5 (62.5)[d]
		Moderate/severe	1 (12.5)	0	0	0	0	1 (12.5)
	2	Any	1 (12.5)	2 (25)	1 (12.5)	0	4 (57.1)	5 (62.5)
		Moderate/severe	0	1 (12.5)	0	0	1 (14.3)	0
Pain on movement	1	Any	4 (50)	1 (12.5)	2 (25)	3 (37.5)	5 (62.5)	6 (75)[e]
		Moderate/severe	0	0	0	0	0	0
	2	Any	3 (37.5)	1 (12.5)	0	1 (12.5)	3 (42.9)	4 (50)
		Moderate/severe	0	0	0	0	2 (28.6)	1 (12.5)
Muscle aches	1	Any	0	2 (25)	1 (12.5)	2 (25)	1 (12.5)	3 (37.5)
		Moderate/severe	0	1 (12.5)	0	0	1 (12.5)	0
	2	Any	0	2 (25)	0	0	0	1 (12.5)
		Moderate/severe	0	0	0	0	0	0
Headache	1	Any	1 (12.5)	1 (12.5)	3 (37.5)	4 (50)	5 (62.5)	1 (12.5)
		Moderate/severe	0	0	2 (25)	0	2 (25)	0
	2	Any	3 (37.5)	3 (37.5)	3 (37.5)	0	2 (28.6)	0
		Moderate/severe	0	2 (25)	3 (37.5)	0	2 (28.6)	0
Fatigue	1	Any	0	1 (12.5)	0	3 (37.5)	4 (50)	2 (25)
		Moderate/severe	0	0	0	1 (12.5)	1 (12.5)	1 (12.5)
	2	Any	0	1 (12.5)	0	0	1 (14.3)	0
		Moderate/severe	0	0	0	0	1 (14.3)	0
Nausea	1	Any	0	0	0	0	3 (37.5)	1 (12.5)
		Moderate/severe	0	0	0	0	1 (12.5)	1 (12.5)
	2	Any	1 (12.5)	0	1 (12.5)	0	2 (28.6)	1 (12.5)
		Moderate/severe	0	0	0	0	0	0
Joint pain	1	Any	0	1 (12.5)	1 (12.5)	0	0	1 (12.5)
		Moderate/severe	0	0	0	0	0	0
	2	Any	0	2 (25)	0	0	1 (14.3)	1 (12.5)
		Moderate/severe	0	0	0	0	0	0

[a] Includes all participants who received ISS alone (two volunteers per ISS concentration).
[b] Only seven participants in this group received the second injection.
[c] $P < 0.001$ for comparison with HBsAg.
[d] $P = 0.03$ for comparison with HBsAg.
[e] $P = 0.04$ for comparison with HBsAg.

Headache was the most commonly reported solicited systemic adverse event but was not associated with any particular study product or dose (Table 1). Fatigue and muscle aches were also commonly reported but again there were no differences between the study groups or doses. Fever (38.9 °C) was reported by one recipient of HBsAg alone with onset >72 h after the second injection; there were no reports of vomiting. Chills (all mild) were reported by one recipient of the 650 μg ISS + HBsAg, 1000 μg ISS + HBsAg, and two recipients of HbsAg (both >24 h after the second dose); diarrhea (none severe) was reported by one each recipient of ISS alone, 300 μg ISS + HBsAg, 1000 μg ISS + HBsAg and two recipients of HbsAg alone; three of the five were reported as moderate and all had onset >24 h after the injection. Three of the systemic adverse events were described as severe including nausea with onset >72 h after the first dose in a recipient of 1000 μg ISS + HBsAg, headache with onset >72 h after the first dose in a recipient of 300 μg ISS + HBsAg, and fatigue with onset >24 h after the first dose in a recipient of 650 μg ISS + HBsAg. All systemic adverse events

resolved within 24 h and none required treatment of medical intervention. There were no differences amongst the groups in unsolicited adverse events.

There were no serious adverse events during the study. One subject withdrew consent after reporting injection site swelling and erythema and moderate disorientation, dizziness, myalgia, and fatigue and mild shortness of breath several hours after the first injection with 1000 μg ISS+HBsAg; all symptoms resolved within 72 h of the injection.

3.2.2. Laboratory abnormalities

Mild abnormalities in clinical chemistries and hematology values were demonstrated in participants during the study; however, there was no discernable pattern or association with a specific study product. Immediately prior to the second dose (56 days after the first dose), 5 (62.5%) of 8 participants in the 3000 μg ISS + HBsAg group had mildly elevated ALT (mean 42.5 u/ml, maximum 72 u/ml; laboratory normal range 5–35 u/ml for females, 10–40 u/ml for males) and AST levels (mean 36.5 u/ml, maximum 61 u/ml; laboratory normal range 5–35 u/ml for females, 10–45 u/ml for males); of these, 3 (37.5%) and 2 (25%) were still mildly elevated 1 week after the second dose. Changes in complement levels or development of antinuclear or anti-DNA antibodies were not observed during the study.

3.3. Antibody response

At baseline, all participants were seronegative for anti-HBsAg antibodies (Table 2). No participants who were immunized with ISS alone developed antibodies against HBsAg. A total of 2 (25%) of the 8 recipients of HBsAg alone (without ISS) developed measurable antibodies (seroresponded) against HBsAg which were only detectable 4 months post-dose 2; neither participant was seroprotected (titer <10 mIU/ml).

All but one participant immunized with ISS + HBsAg responded to HBsAg with measurable antibody levels (a participant immunized with 300 μg ISS + HBsAg). All but two were seroprotected (the 300 μg ISS + HBsAg recipient and a 1000 μg ISS + HBsAg recipient who withdrew from the study after a single dose of vaccine). A statistically significant dose-related trend was demonstrated for both amplitude of the immune response and the rapidity of achieving an immune response (geometric mean titer; $P < 0.001$). At the highest dose level administered (3000 μg ISS + HBsAg), a geometric mean titer of 206.5 mIU/ml was achieved 28 days after the first dose and 1429 and 3045 mIU/ml 7 and 28 days, respectively, after dose 2. At this dose level, 3 (37.5%) of 8 participants were seroprotected 7 days after the first dose which increased to 7 (87.5%) of 8 by 28 days post-dose 1; all participants in the group were seroprotected 7 days after the second dose (Fig. 1). At the next lower dose of ISS + HBsAg (1000 μg), protective responses were not detected in the 7-day post-dose 1 sample but six of the eight participants achieved protective levels by 28 days after the first dose and all seven participants were seroprotected prior to the second dose (the eighth participant withdrew from the study before receiving the second dose). With the two lowest doses of ISS, the majority of participants required a second dose to achieve protective levels.

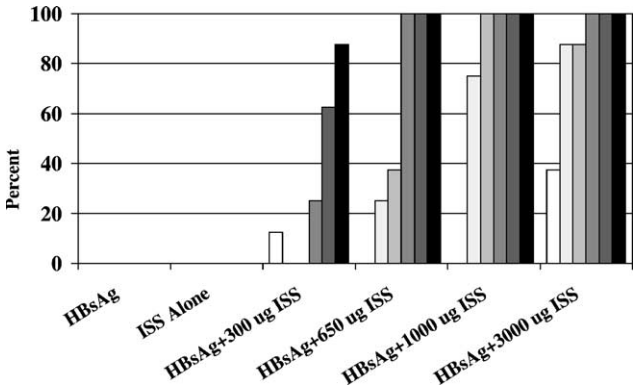

Fig. 1. Proportion of participants achieving a protective antibody level (≥10 mIU/ml) at various time points after immunization with study vaccines. Time points are (by increasing darkness of bar shade) 7 days after dose 1, 28 days after dose 1, 56 days after dose 1 (immediately prior to dose 2), 7 days after dose 2, 28 days after dose 2, 4 months after dose 2.

Table 2
Geometric mean anti-HBsAg antibody titer after each of two doses of HBsAg and/or 1018 ISS and/or HBsAg combined with varying quantities of 1018 ISS in 48 healthy adult volunteers

Time post-immunization	Vaccine geometric mean antibody titer (95% confidence interval)					
	ISS ($N = 8$)	HBsAg ($N = 8$)	HBsAg + 300 μg ISS ($N = 8$)	HBsAg + 650 μg ISS ($N = 8$)	HBsAg + 1000 μg ISS ($N = 8$)	HBsAg + 3000 μg ISS ($N = 8$)
Baseline (pre-dose 1)	1.00 (1.0–1.0)	1.00 (1.0–1.0)	1.00 (1.0,1.0)	1.00 (1.0–1.0)	1.00 (1.0–1.0)	1.00 (1.0–1.0)
7 days post-dose 1	1.00 (1.0–1.0)	1.00 (1.0–1.0)	1.44 (0.61–3.37)	1.00 (1.0–1.0)	1.00 (1.0–1.0)	10.76 (0.53–219.2)[a]
28 days post-dose 1	1.00 (1.0–1.0)	1.00 (1.0–1.0)	1.22 (0.76–1.97)	5.78 (1.72–19.43)	24.75 (4.7–130.4)[a]	206.5 (13.49–3160)[a]
Pre-dose 2 (56 days post-dose 1)	1.00 (1.0–1.0)	1.00 (1.0–1.0)	1.19 (0.79–1.79)	4.27 (1.18–15.47)[b]	46.59 (23.34–93.01)[a]	84.42 (9.87–722.1)[a]
7 days post-dose 2	1.00 (1.0–1.0)	1.00 (1.0–1.0)	2.83 (0.89–9.01)	82.37 (27.45–247.2)[a]	316.1 (133.5–748.8)[a]	1429 (256.8–7954)[a]
28 days post-dose 2	1.00 (1.0–1.0)	1.00 (1.0–1.0)	65.37 (6.63–644.5)[a]	877.6 (326.0–2362)[a]	1545 (689.3–3463)[a]	3045 (641.8–14446)[a]
4 months post-dose 2	1.00 (1.0–1.0)	1.32 (0.86–2.01)	83.24 (16.18–428.2)[a]	756.0 (253.2–2257)[a]	1713 (699.5–4196)[a]	1206 (244.8–5939)[a]

[a] $P < 0.001$ for comparison with ISS alone or HBsAg.
[b] $P = 0.04$ for comparison with ISS alone or HBsAg.

4. Discussion

The results of this phase I study indicate that all dose levels of 1018 ISS+HBsAg vaccine were well tolerated (except in one individual who withdrew consent because of the local and systemic adverse events) and immunogenic in these healthy adults. Although all four ISS + HBsAg doses tested were more immunogenic than HBsAg alone, the 1000 and 3000 μg doses induced rapid and high antibody levels after one or two injections. Injection site adverse events were mostly mild, of short duration, and self-limited, and tended to increase with increasing dose of 1018 ISS; however, adverse events did not increase in frequency with the second dose. Injection site adverse events were reported more frequently in participants who received 1018 ISS compared to those immunized with HBsAg alone whereas systemic adverse events were reported with similar frequency amongst all study participants. Pain at the injection site and limitation of limb motion were the most frequent events, reported in as many as 50–67.5% of participants. Although these rates may appear high, in part this may be a result of the active surveillance involved in a phase I study. Concurrent comparison with a licensed HBV (containing adjuvant) would be informative as these vaccines have an acceptable injection site adverse event profile under routine use. The rates of injection site adverse events in this study were similar to those reported by other investigators with licensed, alum adjuvanted HBV [28] and from this center with a licensed diphtheria–tetanus toxoid vaccine studied using similar surveillance methods [29].

Although a concurrent licensed HBV control would have been ideal, comparison of the antibody responses achieved in this study to those reported with licensed vaccines is less problematic. In this study, 13 (81%) of 16 participants who received 1000 or 3000 μg 1018 ISS demonstrated protective levels of anti-HBsAg antibody 28 days after the first injection and 100% seroprotection after the second dose, using an immunization interval of 2 months. By comparison, licensed HBV is reported to elicit a seroprotective response in up to 20% of healthy young adults after the first dose and up to 71% of recipients 1 month after the second dose with a less effective 1 month interval [28,30–34]. Geometric mean antibody titers with licensed HBV given on a 0, 6 months schedule were reported as 1203 mIU/ml 1 month after the second injection [34] compared to 1545 and 3045 mIU/ml for 1 month after the second 1000 and 3000 μg 1018 ISS doses, respectively, in this study. Geometric mean antibody titers 1 month after a single dose of 3000 μg ISS + HBsAg were 206 mIU/ml compared to 5–27 mIU/ml 1 month after a second injection on a 0, 1 month schedule with licensed HBV [31,35]. High anti-HBsAg antibody titers were maintained 4 months after the second dose of ISS + HBsAg with no decrease in antibody levels in recipients of 300, 650, or 1000 μg ISS + HBsAg and levels still exceeding 1000 mIU/ml in recipients of 3000 μg ISS+HBsAg, despite some decrease in GMT.

Pre-clinical studies demonstrated activity of 1018 ISS in mice, rabbits, dogs, and non-human primates (baboons, cynomolgus monkeys) with induction of a Th1 type of cytokine response in mice [36]. In primate studies with 1018 ISS, similar rates of seroprotection were achieved with a single dose of 1018 ISS + HBsAg [36]. Although in a preliminary communication enhanced immunogenicity of another CpG co-administered with alum-adsorbed HBV has been reported in humans [37], this is the first report in humans using CpG as the sole adjuvant with HBsAg.

The accelerated antibody response and increase antibody titers elicited after one or two doses indicate that the 1018 ISS + HBsAg vaccine may be useful in immunizing difficult to access populations such as adolescents, individuals with high-risk behaviors that increase their risk of hepatitis B, health care workers, and children in developing nations. Other potential uses for a hepatitis B vaccine with enhanced immunogenicity would be populations known to be hypo- or non-responders such as older individuals, renal dialysis patients, and immune compromised hosts [38–41]. The results of this phase I study support further studies with 1018 ISS+HBsAg in healthy adults and hypo- and non-responders to licensed hepatitis B vaccines.

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