CPG 7909 Adjuvant plus Hepatitis B Virus Vaccination in HIV-Infected Adults Achieves Long-Term Seroprotection for Up to 5 Years

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Background. Human immunodeficiency virus (HIV)--infected persons are hyporesponsive to hepatitis B virus (HBV) vaccination. CPG 7909 is an oligodeoxynucleotide containing immunostimulatory CpG motifs that activate human B and plasmacytoid dendritic cells via Toll-like receptor 9. We previously reported that addition of CPG 7909 to a commercial HBV vaccine enhanced the kinetics, magnitude, and longevity of the seroprotective response over 48 weeks. We now report data for the 5-year period following vaccination.

Methods. A randomized, double-blind, controlled trial was conducted to determine clinical safety and immunogenicity of HBV vaccine in adult HIV-infected subjects receiving effective antiretroviral therapy. HBV-susceptible subjects, one-half of whom had experienced previous vaccination failure, were vaccinated at 0, 1, and 2 months with a double adult dose of recombinant HBV vaccine, with or without 1 mg of CPG 7909 (19 subjects per arm). Titers of antibody to HBV surface antigen (anti-HBs) were measured at 6-month intervals for up to 60 months.

Results. The proportion of participants achieving and retaining seroprotection (surface antibody titers, ≥10 mIU/mL) was greater in CPG 7909 recipients (at all time points). Geometric mean anti-HBs titers were higher in the CPG 7909 group than in the control group (without CPG 7909 adjuvant) at all measured time points.

Conclusions. The immunostimulatory properties of CPG 7909 present an important strategy in achieving long-term protection in HIV-infected patients and other HBV vaccine–hyporesponsive populations.

HIV-infected persons have an increased risk of hepatitis B virus (HBV) infection because of shared modes of transmission [1]. Furthermore, if infected with HBV, HIV-infected adults are more likely to become chronically infected [2], to experience progression to cirrhosis, and to develop hepatocellular carcinoma [3, 4]. Thus, there is a need for effective HBV vaccination in this population. By virtue of immunodeficiency, HIV-infected patients are hyporesponsive to vaccination [3–5]. Furthermore, as is the case in immunocompetent populations, seroprotective titers are often not sustained, even if they are initially achieved [6]. CPG 7909 is an oligodeoxynucleotide containing immunostimulatory CpG motifs that directly activate human B cells and plasmacytoid dendritic cells via Toll-like receptor 9 [7]. We have previously reported rapid, higher-titer HBV seroprotection and increased HBV-specific T helper cell response with recombinant HBV vaccine (Engerix-B; GlaxoSmithKline) plus 1 mg of CPG 7909 in immunocompetent populations [8] and in HIV-infected subjects [9]. We now report the long-term seroprotection results with CPG 7909 as an adjuvant to recombinant HBV vaccine in HIV-infected adults.

METHODS

This phase Ib/Ia, randomized, controlled, double-blind vaccine study was conducted at The Ottawa Hospital Clinical Investigation Unit (Ottawa, Canada) with the approval of The Ottawa Hospital Research Ethics Board. Healthy, consenting HIV-infected volunteers (age, 18–55 years) were eligible for enrollment if they...
had received combination antiretroviral therapy for a minimum of 6 months and had CD4 T lymphocyte counts of ≥200 cells/μL and HIV RNA levels of <50 copies/mL for a minimum of 3 months. Subjects tested negative for the presence of antibodies to HBV core antigen, HBV surface antigen (HBsAg), and HBV DNA. Subjects with titers of antibody to HBsAg (anti-HBs) of <10 mIU/mL were randomized to receive recombinant HBV vaccine (Engerix-B; control vaccine group) or vaccine plus CPG 7909 (experimental vaccine group). Randomization was stratified between “naive” subjects (n = 19), with no detectable antibodies or history of previous vaccination, and “hyporesponders” (n = 19), who had received at least 3 previous doses of a commercial HBV vaccine without attaining titers of antibody to HBV surface antigen (anti-HBs) of ≥10 IU/L.

All subjects were vaccinated at 0, 1, and 2 months in a double-blinded fashion. Subjects received 2 intramuscular injections, 1 into each deltoid muscle, of an adult dose of Engerix-B (GlaxoSmithKline); thus, they received a total of 40 μg of HBsAg adsorbed to aluminum hydroxide. Experimental vaccine subjects also received 0.5 mg of CPG 7909 (Coley Pharmaceutical Group) in a 100-μL volume mixed with each injection of vaccine for a total dose of 1 mg of CPG 7909. Blood specimens were collected every 2 weeks for 12 weeks after the first study injection, with clinical follow-up for 12 months for repeated immunologic measurements. Thereafter, serological response was measured at 6-month intervals from month 24 until month 60. This evaluation was nonblinded after month 12.

The proportions of subjects achieving seroprotection (anti-HBs titer, ≥10 mIU/mL) and high-titer seroprotection (anti-HBs titer, ≥100 mIU/mL) were evaluated by the χ² test. For these dichotomous classifications, missing data were imputed if the values before and after were of the same category (i.e., both seroprotective or both sub-seroprotective titers). Once seroprotective titers were lost, this categorization was retained for subsequent time points irrespective of subsequent measurements. Geometric mean anti-HBs titers were compared by repeated-measures/generalized linear mixed models analysis to assess the changes in groups over time. For this analysis, all available data were included in analysis without imputing missing quantitative anti-HBs titers. Time to loss of seroprotection was identified by Kaplan-Meier analysis. All statistical analysis was conducted using SPSS, version 14.0 (SPSS).

### RESULTS

The initial 48-week study was conducted between January 2001 and August 2002. The final (month 60) HBV serology specimen was collected in June 2007. Thirty-eight HBV-susceptible subjects (19 were HBV seronegative and vaccine naive; 19 experienced HBV vaccine failure) were evaluated (table 1). Clinical and laboratory safety, tolerance, and serological efficacy results for the initial 48 weeks of this study were previously reported [9]. Seroprotective titers were higher and achieved more rapidly in recipients of vaccine plus CPG 7909.

By passive surveillance, no clinical adverse events that could be attributed to vaccine and/or CPG 7909 were identified between months 12 and 60 of follow-up. The difference in seroprotection (≥10 mIU/mL) between study arms remained significant (P < .05) at all time points from month 24 to month 60 (figure 1). Similar trends were seen for CPG 7909 versus controls when compared within the subgroups of vaccine-naive subjects and subjects with prior vaccine hyporesponder (data not shown). Addition of CPG 7909 to vaccine resulted in more subjects achieving high anti-HBs titers (≥100 mIU/mL) as early as week 8 (68% of experimental group subjects vs. 26% of control group subjects; P = .02). These high titers were maintained from month 24 (67% vs. 17%) to month 54 (43% vs. 12%; P < .05; data not shown). The time to loss of seroprotective titers varied between groups as well (figure 2).

### Table 1. Characteristics of hepatitis B virus (HBV)-susceptible subjects randomized to receive recombinant HBV vaccine with or without CPG 7909 adjuvant.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vaccine (n = 19)</th>
<th>Vaccine plus CPG 7909 (n = 19)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean years ± SD</td>
<td>42.9 ± 7.3</td>
<td>41.0 ± 7.4</td>
<td>NS²</td>
</tr>
<tr>
<td>Male sex, no. of patients</td>
<td>18</td>
<td>14</td>
<td>NS²</td>
</tr>
<tr>
<td>Anti-HBs at screening, median mIU/mL (range)</td>
<td>0 (0–4)</td>
<td>0 (0–2)</td>
<td>NS²</td>
</tr>
<tr>
<td>CD4 T cell count, mean cells/μL ± SD</td>
<td>543 ± 228</td>
<td>647 ± 262</td>
<td>NS⁴</td>
</tr>
</tbody>
</table>

NOTE. HBV-susceptible subjects were defined as having no serological evidence of prior natural exposure to HBV and having never received HBV vaccine or having received at least 3 previous doses of a HBV vaccine without attaining titers of antibody to HBV surface antigen (anti-HBs) of ≥10 IU/L.

NS, not significant.

a By t test.
b By χ² test.
c By 2-tailed Mann-Whitney U test.
DISCUSSION

We have already reported the early results of this randomized, controlled, long-term study [9]. In this work, we demonstrated that CPG 7909 as a vaccine adjuvant with recombinant HBV vaccine is safe and well tolerated in HIV-seropositive adults receiving effective anti-HIV therapy and that it significantly enhanced the kinetics and magnitude of the anti-HBs response. We have also published similar findings about healthy HIV-negative adult volunteers receiving recombinant HBV vaccine plus CPG 7909 adjuvant [10]. It is well accepted that antibodies alone are sufficient for protection against HBV infection, and an antibody level $\geq 10$ mIU/mL is considered protective.

We report that the enhanced response in vaccinated, HIV-
seropositive subjects was durable, with significantly more CPG 7909 recipients than control subjects maintaining seroprotective anti-HBs titers for up to 60 months. This was achieved both in vaccine-naive participants and in those who had previously experienced vaccine failure.

The importance of achieving HBV seroprotection in vaccine-naive, HIV-seropositive persons is obvious given risk behaviors in this population that increase the probability of exposure to HBV. Furthermore, the risk for serious long-term complications from HBV infection is greater in those coinfected with HIV. Therefore, the importance of preventing HBV infection is clear. The demonstrated durability of seroprotective anti-HBs titers addresses the issue of waning seroprotection in vaccine-hyporesponsive populations, including those infected with HIV. It remains an issue of debate as to what degree of protection vaccine-hyporesponsive persons retain against HBV in this context. However, standard philosophy is that lower antiHBs titers predict reduced protection from natural HBV infection. One strategy to address this issue is to serially monitor antibody levels in at-risk vaccine recipients and provide booster vaccines each time antiHBs titers fall below 10 mIU/mL [11]. In practice, this approach is difficult to implement, because of issues related to cost, personnel, and loss to follow-up. Therefore, the durability of the titers generated by using CPG 7909 adjuvanted vaccine in the present study represents an important advance. At the very least, the frequency of booster dosing to maintain seroprotective titers may be reduced by use of this adjuvant, given the longer time noted for decay of antibody titers.

This study was conducted in a population of otherwise healthy HIV-infected participants receiving HAART, with maximal suppression of HIV load and relatively high CD4 T cell counts. Safety and efficacy evaluation would be valuable for persons with a higher degree of immune compromise and for those not receiving antiretroviral therapy. Nevertheless, on the basis of these promising data, we believe that the use of hepatitis B vaccine containing a Cpg oligodeoxynucleotide adjuvant represents an important advance in achieving long-term seroprotection against HBV infection.

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Potential conflicts of interest. C.L.C. and D.W.C. are former investigators and consultants with Coley Pharmaceutical Group; J.B.A. is a former investigator with Coley Pharmaceutical Group; H.L.D. is an employee of Coley Pharmaceutical Group. I.S.: no conflicts.

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