Granulocyte Macrophage Colony–Stimulating Factor as an Adjuvant for Hepatitis B Vaccination of Healthy Adults


Granulocyte macrophage colony–stimulating factor (GM-CSF) has shown promise as an adjuvant to improve the kinetics and magnitude of the immune response after vaccination. It was hypothesized that GM-CSF given intramuscularly (IM) with hepatitis B vaccine would result in increased seroconversion rates and antibody titers. In total, 108 healthy volunteers (18–45 years old) received recombinant hepatitis B vaccine IM at 0, 1, and 6 months and were randomized to receive either concurrent GM-CSF (80 or 250 mg) or placebo IM with the first two vaccinations. The percentages of subjects achieving a protective level of antibody at day 56 were 58.3%, 58.8%, and 58.3% in the placebo and 80- and 250-mg GM-CSF arms, respectively. The geometric mean titers of antibody measured on days 28, 56, and 189 were not statistically different between arms. GM-CSF given immediately before recombinant hepatitis B vaccination was safe and well tolerated but did not appear to provide significant adjuvant activity at this dose.

Hepatitis B is a serious worldwide infection that is mostly preventable by vaccination. Hepatitis B vaccine given as three intramuscular (IM) injections at 0, 1, and 6 months results in ~90% of healthy adults achieving a protective antibody level of ≥10 mIU/mL. The long-term risk of hepatitis B infection is inversely related to the maximal antibody titer achieved by vaccination [1]. However, because of many factors, ~40%–50% of vaccinees fail to receive the third 6-month injection [2, 3]. The resultant 2-dose regimen induces insufficient antibody to provide immediate seroprotection (>10 mIU/mL) in many healthy adults and does not result in the high-titer antibody associated with long-term protection (>1000 mIU/mL). Strategies to produce an earlier seroconversion/seroprotection rate to hepatitis B vaccine would be highly desirable, especially in those at immediate risk, such as health care workers or seronegative sex partners of seropositive persons. Studies performed to improve the kinetics of response have included the use of higher vaccine dose, intradermal administration, an accelerated dosing schedule, and a modification of the adjuvant used with vaccination (currently alum) [4]. Although dozens of new compounds have been investigated for adjuvant activity in animal and human vaccine trials in the past decade, an increasing emphasis has been given to the possibility of using specific cytokines to elicit vaccine responses, rather than using non-specific formulations that induce these cytokines [5].

Granulocyte macrophage colony–stimulating factor (GM-CSF) is a potent vaccine adjuvant for improving both humoral and cellular immune responses [6, 7]. The potential mechanisms of its adjuvant effects are likely on the antigen-presenting cells (APCs) and include up-regulation of major histocompatibility class II [8] and costimulatory molecules, such as B7, and enhanced differentiation of precursors into mature dendritic cells [9]. This last effect is of special interest, since dendritic cells are critical for responses to neoantigens.

We hypothesized that GM-CSF given in a paracrine fashion with hepatitis B IM immunization on days 0 and 28 would achieve a higher rate of seroconversion/seroprotection after two immunizations. Long-term immunity secondary to increased titers of antibody to surface antigen might therefore also be improved. Thus, a placebo-controlled, double-blinded, randomized trial of the safety and reactogenicity of IM GM-CSF given locally as an adjuvant for hepatitis B vaccination was undertaken.

Methods

Study drug and dose. Yeast-derived recombinant human GM-CSF (sargramostim; leukaene) or identical placebo diluent was supplied by Immunex. Recombinant hepatitis B vaccine (Recombivax HB; Merck, West Point, PA) was used at a dose of 10 μg of hepatitis B surface antigen (HBsAg)/1.0 mL.
Study population. Healthy adults ages 18–45 years who were HBs IgG antibody negative were enrolled.

Study design. Subjects were randomized to receive either GM-CSF (80 or 250 µg) or placebo IM immediately before (5 min) IM administration of recombinant hepatitis B vaccine. The study drug was administered IM in the same deltoid muscle at the same depth at the 0- and 1-month immunizations. All participants received the third hepatitis B vaccine alone at 6 months. General symptoms, such as malaise, myalgia, headache, fever, and nausea, along with vaccination site pain, tenderness, erythema, and edema were recorded on a severity scale of 0 (none) to 3 (severe). Systemic complaints and local examination of the injection site for the presence of erythema, induration, and local adenopathy were recorded on days 1, 28, 56, and 189. A complete blood count with differential was also obtained on days 1 and 29.

Laboratory methods. Anti-HBs antibody IgG was measured by commercial RIA (AUSAB; Abbott Laboratories, Abbott Park, IL), and titers were determined in milli-international units per milliliter and compared with a standard immunoglobulin preparation (WHO). Sera with antibody titers ≥1 mIU/mL were considered positive; an anti-HBs titer ≥10 mIU/mL was considered protective. For determination of geometric mean titers (GMTs), all unmeasurable titers were given a value of 1.

Statistical considerations. The size of the study population was determined on the basis of the likelihood of having protective titers after the first dose of vaccine. Assuming a 15% response rate in the placebo arm and a 45% response rate in the GM-CSF arm in young healthy adults, the study had an 80% power to detect this difference by use of a two-sided test. Differences in the placebo arm and a 45% response rate in the GM-CSF arm in young healthy adults, the study had an 80% power to detect this difference by use of a two-sided test. Differences in the

<table>
<thead>
<tr>
<th>Study arms</th>
<th>Placebo (n = 36)</th>
<th>80 µg (n = 36)</th>
<th>250 µg (n = 36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>16</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Caucasian</td>
<td>34</td>
<td>33</td>
<td>34</td>
</tr>
<tr>
<td>African American</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Other ethnicity</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Age range (mean)</td>
<td>18-45 (27.7)</td>
<td>18-45 (28.7)</td>
<td>18-43 (28.4)</td>
</tr>
<tr>
<td>No. of volunteers with protective titer at each time pointa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 28 (n = 107)</td>
<td>2 (36)</td>
<td>6 (35)</td>
<td>4 (36)</td>
</tr>
<tr>
<td>Day 56 (n = 106)</td>
<td>21 (36)</td>
<td>20 (34)</td>
<td>21 (36)</td>
</tr>
<tr>
<td>Day 189 (n = 102)</td>
<td>32 (34)</td>
<td>33 (33)</td>
<td>34 (35)</td>
</tr>
</tbody>
</table>

a HBsAb titer ≥10 mIU/mL

Results

Subjects. In total, 131 volunteers completed the initial screening and completed the protocol-indicated screening examinations. Of these, 108 were found to be eligible and were randomized in a double-blind manner (table I). Of the 108 subjects, 102 completed the entire study. Four volunteers were lost to follow-up because of relocation. One volunteer refused further vaccination because of development of a headache of moderate intensity the day after vaccination that persisted for 1 week. She was able to work and perform all functions of daily living. One volunteer was withdrawn because of a high-risk exposure to an HBsAg-positive carrier that required immediate hepatitis B immunoglobulin prophylaxis.

Safety and side effects. Side effects in the three arms were few, generally mild, and evenly distributed in the placebo versus GM-CSF arms. The most common symptom was mild pain and tenderness at the site of injection. Two subjects developed moderate pain at the injection site requiring medication 1 day after the second injection. No participant had any incapacitating symptoms that required bed rest or resulted in loss of work or cancellation of social activities.

GM-CSF administration increased the white blood cell (WBC) and total neutrophil counts. There was no significant difference between the three arms before vaccination, but after the first vaccination the WBC count in the 250-µg GM-CSF arm was significantly greater than in the placebo recipients (7000 vs. 6000/µL, P = .02). This increase was even greater on the day after vaccination two (80 µg vs. placebo, 6600 vs. 6100/µL, P = .3; 250 µg vs. placebo, 7300 vs. 6100/µL, P = .009). The same effect of the GM-CSF was observed on the neutrophil percentage (250 µg vs. placebo, 63.8% vs. 57.3%, P = .006).

Immunogenicity: HBs antibody was measured on days 0, 28, 56, and 189. The number of volunteers protected (HBsAb titer ≥10 mIU/mL) at each time point was not significantly different. A trend toward an increase in protective titers, 10 of 71 versus 2 of 36, was seen with GM-CSF 28 days after the first vaccination (table I). A comparison of the GMT of HBs antibody in the three study arms failed to show any statistically significant difference in titers (figure I). Three of 102 volunteers who completed the study did not seroconvert: 1 in the 250-µg arm and 2 in the placebo arm.

Discussion

Hepatitis B vaccination has led to a marked reduction in the incidence of new infections with hepatitis B worldwide and is even leading to a reduction in new cases of hepatoma [10]. However, a strategy that would overcome the need to use three doses of recombinant vaccine to achieve high-titer antibody would be an important advance. A shorter vaccination course would lead to a marked decrease in the expense of immunization and would potentially lead to earlier protection of persons who have ongoing risk at the time they present for vaccination. Because improvement in the kinetics and magnitude of antibody response were seen in initial human vaccination trials in which GM-CSF was used as an adjuvant [6, 11], we conducted a large study to determine whether such a strategy would be effective in healthy adults.

We administered the GM-CSF immediately before and at the same site and same depth as the subsequent recombinant hepatitis B vaccine. In the 108 subjects enrolled in this study, we
were unable to measure an appreciable immunologic benefit by the paracrine administration of either 80 or 250 μg of GM-CSF. The IM inoculation of GM-CSF was extremely well tolerated, with few moderate and no severe reactions, and with little difference between the GM-CSF and placebo arms of the trial. The physiologic activity of GM-CSF given by this route was apparent, in that a dose-dependent increase in the WBC count was seen after administration.

The GM-CSF dose used in this study was determined on the basis of results of two earlier studies. In the first (Tarr et al. [6]), *Escherichia coli*-derived GM-CSF led to an improvement in the number of healthy adults who responded to a single hepatitis B vaccination using a different product (Engerix) than used here. In that study, three doses of GM-CSF (20, 40, and 80 μg) were compared when given concomitantly via three different routes (intradermal, subcutaneously, and IM). The number of seroprotected subjects after a single immunization (n = 27, each group) were 0 in the placebo arm, 0 in the intradermal arm, 6 by the subcutaneous route, and 5 in the IM arm. A tendency toward an improvement with lower doses of GM-CSF was noted. However, in a trial of influenza vaccination combined with yeast-derived GM-CSF, an improved response was seen in the higher dose arm than in the lower dose arm (250 vs. 125 μg; Heuser MD, Schilling ML, Powers DC, et al., unpublished data). The IM route for GM-CSF used in this study was chosen on the basis of the greater experience and on the data set available when hepatitis B vaccine is given IM.

The lack of an adjuvant effect of GM-CSF in this trial may have been due to number of factors. First, in murine models of DNA vaccines, administration of the GM-CSF as a fusion molecule on the same plasmid or as coadministration on a separate plasmid resulted in impressive adjuvant activity [12]. Whether admixing the GM-CSF directly into the vaccine, which is effective in animal models [13], would improve efficacy is not known. Second, the possible negative effects of alum in the vaccine preparation were not explored here. Third, it is possible that the kinetics of the GM-CSF response are such that the dose must be given either before or on multiple occasions before vaccination [7]. In earlier studies, the use of an IgG GM-CSF fusion protein or of liposome-encapsulated GM-CSF, which had a longer half-life, was more effective than soluble GM-CSF alone [14]. Fourth, it is possible that the dose we chose was either too high and led to counterregulatory signals, as suggested by Tarr et al. [6], or too low, as suggested by the influenza study. The exact local dose needed in humans to achieve improved APC function is also not known; however, our dosing regimen clearly resulted in systemic effects as measured by increasing WBCs. Fifth, it is possible that the immunogenicity of hepatitis B vaccine is such that the amount of costimulatory molecules on the presenting cell is not critical for the magnitude of the response. It is possible that GM-CSF would still be useful when used in a paracrine or admixed fashion when applied with an immunogen that required class I presentation, such as attenuated viruses, nucleic acid vaccines, or vector-based systems, such as poxvirus constructs [15].

In conclusion, GM-CSF when given at the doses studied here and administered at the site of the subsequent hepatitis B vaccination did not lead to a clinically significant effect. Further studies using multiple dosing regimens, longer-acting preparations of GM-CSF, or admixtures of the protein or nucleic acid constructs will be needed to determine the role of GM-CSF as an adjuvant in human immunization.

References


