Nonresponsiveness to Hepatitis B Vaccine in Health Care Workers

Results of Revaccination and Genetic Typings


Twenty-eight health care workers who had a poor antibody response when initially vaccinated with hepatitis B vaccine were revaccinated with three additional 20-μg doses. Eight of the twenty nonresponders, who had levels of antibody to hepatitis B surface antigen (anti-HBs) of less than 8 estimated radioimmunoassay (RIA) units, and all 8 of the hyporesponders, who had anti-HBs levels of 8 or 16 RIA units, attained anti-HBs levels of 36 RIA units or more after revaccination. Tests for HLA-A, B, C, and DR; for complement proteins C2, C4A, C4B, and BF; and for the erythrocyte enzyme glyoxalase I were done in 17 nonresponders and 3 hyporesponders. Nine (45%) had HLA-DR7 and 8 (40%) had HLA-DR3, compared with an expected rate of 23% in the general population. At least one of two extended haplotypes (B44, DR7, FC31 or B6, DR3, SC01) were detected in 6 of the 9 who did not respond to revaccination, compared with 2 of 11 who responded to a second course of vaccine. Poor responders to vaccine may benefit from revaccination, and genetic factors may modulate the immune response to vaccination.

HEPATITIS B VIRUS infection is an occupational hazard for health care workers who have long-term or continuous exposure to human blood (1-4). Many of the infections caused by hepatitis B virus are subclinical and not associated with an easily identifiable source of infection. Therefore, and because prophylaxis with hepatitis B immune globulin after exposure is of limited effectiveness in preventing hepatitis B virus infection (5, 6), efforts have been directed at active immunization with hepatitis B vaccine before exposure (7-10).

Studies of hepatitis B vaccine in health care personnel and other high-risk groups indicate that the vaccine is safe, effective, and immunogenic (7-10). In each of the controlled trials of plasma-derived hepatitis B vaccine in the United States, a small group of participants has either failed to respond or responded feebly (achieving low levels of antibody) (7-10). In the Boston Inter-Hospital Hepatitis B Vaccine Study, 666 health care workers were vaccinated, and 28 (4.2%) had absent or poor antibody responses (9).

This study examined factors related to vaccine nonresponsiveness in health care workers. Demographic characteristics, response to revaccination, indicators of immune responsiveness, and genetic markers were evaluated. Our findings suggest that genes present in the major histocompatibility complex may modulate the immune response to hepatitis B vaccine and that health care personnel who have low antibody levels or no response to an initial course of hepatitis B vaccine may benefit from revaccination.

Methods

PARTICIPANTS

All the volunteers in this study were health care workers who had been vaccinated with three 20-μg doses (doses one to three) of hepatitis B vaccine (Heptavax B; Merck Sharp & Dohme, West Point, Pennsylvania) intramuscularly in the deltoid region at 0, 1, and 6 months, as previously reported (9). Nonresponders and hyporesponders were defined by the level of antibody achieved. Each person was revaccinated with three 20-μg doses of vaccine intramuscularly in the deltoid region (doses four to six) at 0, 1, and 6 months. The mean (±SD) interval between primary vaccination and revaccination was 18 ± 4 months (range, 9 to 25). Informed consent was obtained from each participant, and the protocol was approved by the Institutional Review Board at Boston City Hospital.

LABORATORY METHODS

Levels of antibody to hepatitis B surface antigen (anti-HBs) were quantified by calculating estimated radioimmunoassay (RIA) units (AUSAB; Abbott Laboratories, North Chicago, Illinois). Forty estimated RIA units have been shown to be equivalent to approximately 10 mIU of anti-HBs (Miller WJ, Merck Sharp & Dohme Research Laboratories).

Nonresponders were defined as persons who had anti-HBs levels of less than 8 RIA units after doses one to three of Heptavax B. Hyporesponders were persons who had anti-HBs levels of 8 or 16 RIA units after three doses of vaccine. Persons who achieved anti-HBs levels of 36 RIA units or more after revaccination with doses four to six were defined as ultimate responders. Those who had 16 RIA units or less of anti-HBs after revaccination were defined as absolute nonresponders.

Assessment of T4/T8 ratios and total lymphocyte counts were done as previously described (11). Intradermal skin tests were done with mumps antigen (Eli Lilly Co., Indianapolis, Indiana) and Candida albicans (trichophyton B; Hollister-Stier, Inc., Spokane, Washington) according to the manufacturers' recommendations.

GENETIC TYPING

Human leukocyte antigens (HLA) A, B, C, and DR were determined by a microlymphocytotoxicity assay (12, 13). Alleles of C2, C4A, C4B, and factor B (BF) were determined by isoelectric focusing on polyacrylamide gels or agarose gel elec-
trophoresis, as previously described (14-16). Typing of the erythrocyte enzyme glyoxalase I was done with cellulose acetate electrophoresis and specific enzyme reagents (17). The nomenclature for genetic polymorphism of BF, C2, and C4 has been discussed previously (18) and is designed to conform with the International System for Human Gene Nomenclature (19). Nomenclature for complotypes and extended HLA/complemen haplotypes has been described by Awdeh and coworkers (18).

Results

The 631 responders to the initial three doses of hepatitis B vaccine did not significantly in age, sex, or race from the 8 hyporesponders and the 20 nonresponders (Table 1). In addition, there were no significant differences between the three groups in country of origin; time in occupation; recent, identified exposure to hepatitis B virus; recent transfusion; or occupational category in the hospital.

REVACCINATION

All eight hyporesponders had appreciable levels of antibody to hepatitis B surface antigen (anti-HBs) after revaccination (Table 2). Six of the eight responded to the fourth dose of vaccine, and all eight responded after dose five, but the geometric mean titer of anti-HBs was less than one tenth that of the responder group. Titers of anti-HBs of 36 radioimmunoassay (RIA) units or more were sustained for a 9-month period after revaccination.

Five of the twenty nonresponders developed anti-HBs after the fourth dose of vaccine, with a geometric mean titer of 216 RIA units (range, 54 to 1600). Antibody appeared in 3 more nonresponders after the fifth injection. Thus, 8 of the 20 nonresponders had a geometric mean titer of anti-HBs of 100 RIA units (range, 36 to 352). All 20 were given the sixth dose of vaccine, and 1 additional person seroconverted (54 RIA units) after dose six; however, 6 of the 12 seronegative persons were lost to follow-up after the sixth dose of vaccine. Thus, 9 of 20 nonresponders became ultimate responders, but the geometric mean titer of anti-HBs was lower than that in initial responders to vaccine and hyporesponders who were revaccinated. In addition, 2 of the 9 nonresponders who responded to revaccination subsequently became seronegative during the next 9 months of follow-up.

IMMUNOLOGIC STUDIES

Because there are no indicators of specific cellular im-

Table 1. A Comparison of the Characteristics of Responders, Hyporesponders, and Nonresponders to Initial Vaccination with Hepatitis B Vaccine

<table>
<thead>
<tr>
<th></th>
<th>Responders (n = 631)</th>
<th>Hyporesponders (n = 8)</th>
<th>Nonresponders (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>31.1 ± 8.3</td>
<td>39.4 ± 11.7</td>
<td>38.6 ± 11.4</td>
</tr>
<tr>
<td>Men/women</td>
<td>328/303</td>
<td>5/3</td>
<td>10/10</td>
</tr>
<tr>
<td>White race</td>
<td>593</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td>Anti-HBs, RIA units</td>
<td>≥ 36</td>
<td>8 or 16</td>
<td>&lt; 8</td>
</tr>
</tbody>
</table>

*Age is given as mean ± SD. Anti-HBs = antibody to hepatitis B surface antigen (in estimated radioimmunoassay [RIA] units).

Table 2. Response of Hyporesponders to Revaccination with Hepatitis B Vaccine

<table>
<thead>
<tr>
<th>Vaccine Dose</th>
<th>Cumulative Seroconverters* (n = 8)</th>
<th>Anti-HBs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RIA units</td>
<td>Geographic Mean Titer</td>
</tr>
<tr>
<td>n</td>
<td>n</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>628</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>208</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>621</td>
</tr>
</tbody>
</table>

* The number of persons who had had seroconversion up to and including each dose. Seroconversion was defined as an anti-HBs (antibody to hepatitis B surface antigen) level of ≥ 36 estimated radioimmunoassay (RIA) units.

Humoral function to hepatitis B virus, we looked at nonspecific markers of cellular immune competence in the study group. Eleven of the twenty-eight volunteers consented to cutaneous testing of delayed hypersensitivity with mumps or Candida antigens, and 10 of them responded to at least one of the antigens. The mean ratio of helper (T4) to suppressor (T8) lymphocytes was examined in 4 of the 8 hyporesponders and in 11 of the 20 nonresponders. The mean ratios (± SD) in the hyporesponders (1.8 ± 0.6) and nonresponders (1.6 ± 0.9) were indistinguishable from the mean ratio in 41 immunocompetent adults (1.9 ± 0.7) who responded to hepatitis B vaccine (11). Furthermore, no differences in mean T4/ T8 ratios were detected between ultimate responders (1.8 ± 0.6) and absolute nonresponders to vaccine (1.7 ± 0.8).

HISTOCOMPATIBILITY TESTING

Human leukocyte antigen (HLA) A, B, C, and DR alleles and genetic variants of serum complement proteins factor B (BF), C2, C4A, and C4B as well as glyoxalase I were measured in 17 nonresponders and 3 hyporesponders (Table 3). The frequencies of HLA-DR3 and DR7 among the 20 white nonresponders and hyporesponders were higher than the frequencies expected in white persons in the general population.

Ten of the seventeen nonresponders had a single DR antigen (Table 3), which may reflect a homozygous DR allele or one DR allele and a blank (-). The frequency of DR3/3 or DR3/- and DR7/7 or DR7/- were at least fivefold higher among nonresponders and hyporesponders than among the control group. In the absence of family studies, it is difficult to discriminate between homozygous DR alleles and a single DR allele with a blank.

Genes of the four complement components, glyoxalase I, and HLA alleles A, B, C, and DR are closely linked within the major histocompatibility complex. Although in many instances there is a random distribution of complotypes among the different HLA-A, B, and DR alleles, in other cases HLA-A, B, and DR alleles segregate and are in linkage disequilibrium with certain complotypes (18). Alleles for HLA-B8 and DR3 are frequently in linkage disequilibrium with complotype SC01, which contains the S allele of BF, the C variant of C2, the O (QQ or null) allele of C4A, and the I variant of C4B. Similarly, HLA-B44 and DR7 are often in linkage disequilibrium with the complotype FC31. Such HLA-B,
Table 3. Major Histocompatibility Complex Markers in Hyporesponders and Nonresponders to Initial Hepatitis B Vaccination

<table>
<thead>
<tr>
<th></th>
<th>Hyporesponders (n = 3)</th>
<th>Nonresponders (n = 17)</th>
<th>Hyporesponders and Nonresponders (n = 20)</th>
<th>Ultimate Responders (n = 11)</th>
<th>Absolute Nonresponders (n = 9)</th>
<th>Controls* (n = 320)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA antigen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR3†</td>
<td>1 (33)</td>
<td>7 (41)</td>
<td>8 (40)</td>
<td>3 (27)</td>
<td>5 (55)</td>
<td>75 (23)</td>
</tr>
<tr>
<td>DR7</td>
<td>2 (67)</td>
<td>7 (41)</td>
<td>9 (45)</td>
<td>5 (45)</td>
<td>4 (44)</td>
<td>72 (23)</td>
</tr>
<tr>
<td>DR3/7</td>
<td>1 (33)</td>
<td>2 (12)</td>
<td>3 (15)</td>
<td>1 (9)</td>
<td>2 (22)</td>
<td>29 (9)</td>
</tr>
<tr>
<td>DR3/3 or 3/-</td>
<td>0</td>
<td>5 (29)</td>
<td>5 (25)</td>
<td>2 (18)</td>
<td>3 (33)</td>
<td>12 (4)</td>
</tr>
<tr>
<td>DR7/7 or 7/-</td>
<td>0</td>
<td>3 (18)</td>
<td>3 (15)</td>
<td>1 (9)</td>
<td>2 (22)</td>
<td>11 (4)</td>
</tr>
<tr>
<td>DRX/X or X/-†</td>
<td>1 (33)</td>
<td>2 (12)</td>
<td>3 (15)</td>
<td>2 (18)</td>
<td>1 (11)</td>
<td>58 (18)</td>
</tr>
</tbody>
</table>

Markers of extended haplotypes

|                         |                        |                        |                                          |                               |                               |                     |
| HLA-B8, DR3, SC01       | 1 (33)                 | 4 (24)§                | 5 (25)                                  | 1 (9)                         | 4 (44)§                       | 51 (17)             |
| HLA-B44, DR7, FC31      | 0                      | 4 (24)                 | 4 (20)                                  | 1 (9)                         | 3 (33)                        | 19 (6)              |

* Controls consisted of healthy white persons in the general population.
† One hyporesponder and two nonresponders had both HLA-DR3 and DR7. Both nonresponders did not have detectable antibody to hepatitis B surface antigen after six doses of vaccine.
§ DRX is any DR specificity other than DR3 or DR7.
¶ One nonresponder had both extended haplotypes and did not have detectable antibody to hepatitis B surface antigen after six doses of vaccine.

HLA-DR, and complotype sets in significant positive linkage disequilibrium are called extended haplotypes. Markers of two extended haplotypes—HLA-B8, DR3, SC01 and HLA/B44, DR7, FC31—were found in 1 of the 3 hyporesponders and in 7 of the 17 nonresponders (Table 3); 1 of the 7 nonresponders has both extended haplotypes. Of note, only 2 of 11 of the ultimate responders had one of these extended haplotypes, compared with 6 of the 9 absolute nonresponders. One absolute nonresponder had both extended haplotypes.

Discussion

Hepatitis B vaccine is composed of purified hepatitis B surface antigen (HBsAg) prepared from the plasma of persons who are chronic HBsAg carriers. Several factors are known to be associated with diminished antibody responsiveness to vaccination. The immune response is excellent in infants, children, and young adults but decreases with advancing age (20). Patients with renal failure or immunosuppression have a poor humoral response to vaccination (21, 22). Nonresponsiveness has also been attributed to freezing the vaccine before administration (8, 23) and to injection of the vaccine into the buttock rather than the gluteal region (24).

Vaccine nonresponders in this study were healthy health care workers who originally had participated in the Boston Inter-Hospital Hepatitis B Vaccine Study (9), in which the overall response rate was 96% and the geometric mean titer of anti-HBs was 6300 RIA units. More than 79% of the responders to hepatitis B vaccine developed levels of anti-HBs of 1000 RIA units, or more, and more than 43% had levels that exceeded 100 000 estimated RIA units (9). Although responders to the vaccine in our study were younger than nonresponders and hyporesponders, the differences were not statistically significant. All injections of vaccine were given intramuscularly in the deltoid region, and the vaccine was stored according to the manufacturer’s instructions. All of our subjects with poor responses to hepatitis B vaccine, as well as those in another study (25), had no obvious defect in delayed skin test hypersensitivity or altered ratios of T-cell phenotypes.

Because of previous information indicating genetic regulation of the immune response to HBsAg in mice (26, 27) and because data in humans are limited (28), we investigated possible correlations between HLA type and poor responsiveness to hepatitis B vaccine. Antigens for the HLA types are encoded by genes within a region of the short arm of chromosome 6 known as the major histocompatibility complex. The functions of this complex, including antigen presentation and immune regulation, are carried out by two classes of glycoproteins (29). Class I proteins are important for the recognition of cell surface antigens and are associated with beta-2-macroglobulins in virtually all cell membranes. Class I proteins include the H-2K and D molecules in mice and HLA-A, B, and C molecules in humans (29). Class II proteins control responsiveness to soluble antigens and are present on a limited number of cell types. Class II proteins include the H-2Ia molecules in mice and the HLA-DR molecules in humans (29).

Our data show a higher than expected frequency of DR3 (40%) and DR7 (45%) among the 20 white hyporesponders and nonresponders, compared with a 23% rate for each allele in the white control group. Walker and colleagues (28) have reported a higher than expected frequency of HLA-DR7 among homosexual men who responded poorly to initial vaccination with hepatitis B vaccine (61% compared with 20%, p < 0.001). Hyporesponders and nonresponders in our study also had a higher than expected frequency of single HLA-DR7 and DR3 alleles.

Genetic factors have been associated with increased host susceptibility to Reiter’s disease, ankylosing spondylitis, chronic lymphocytic leukemia, and “autoimmune” chronic active hepatitis, among others (29). Mackay and Tait (30) reported an increased frequency of HLA-DR3 in 48 patients with autoimmune chronic active hepatitis and a high degree of concurrence between HLA-B8 and DR3. Our data suggest that increases in HLA-DR3 and DR7 may be the result of increases in two extended haplotypes—HLA-B8, DR3, SC01 and...
HLA-B44, DR7, FC31—among persons with a poor response to hepatitis B vaccine. Further studies comparing the frequency of extended haplotypes in high responders and nonresponders should verify these results.

Studies of genetic regulation of the humoral response to HBsAg in the murine model (26, 27) indicate that T-dependent antibody responses to the common a and subtype d and y determinants are regulated by genes that map in the murine major histocompatibility complex; additional data suggest a hierarchy of responsiveness to HBsAg related to different H-2 haplotypes in mice. Presently, it is not known if genetic factors contribute to differences in clinical presentation and outcome of hepatitis B virus infection. Although our data and those of others (26-28) suggest a role for genetic modulation of the immune response to hepatitis B vaccine, paradoxically, no increased frequency of chronic infection is seen among vaccine nonresponders who become infected with hepatitis B virus (31).

Cellular mechanisms responsible for host nonresponsiveness to HBsAg or hepatitis B vaccine are not well delineated. The immune response to a particular antigen, such as HBsAg, may fail because macrophages do not process the antigen properly for the T cells or because Ia/DR determinants for antigen presentation to the T cell may be lacking in specific T-cell populations. Likewise, B cells may lack proper receptors for T-cell interaction or may be unable to produce antibody in response to specific antigen stimulus (29).

All 8 responders and 9 of the 20 nonresponders in our study developed levels of anti-HBs of 36 RIA units or more after revaccination. Because 6 nonresponders were lost to follow-up after receiving dose six, the response rate to revaccination could range from 45% to 65%. By comparison, Kalish and coworkers (25) have reported a 20% rate of nonresponsiveness among 200 homosexual men initially vaccinated with hepatitis B vaccine. As noted in our study, the response rate was higher for responders than nonresponders. However, Kalish and coworkers (25) found that the poor responses to vaccination in homosexual men were correlated with a higher frequency of IgM antibody against cytomegalovirus, as well as with increased numbers of sexual contacts and more sexually transmitted diseases, than in homosexual men who responded.

Although all of the responders and approximately half of the nonresponders in our study ultimately responded to revaccination, levels of anti-HBs achieved in these groups were less than one tenth of those in initial responders. Furthermore, two of the initial nonresponders who ultimately responded lost antibody during the 9 months of follow-up, suggesting that levels of antibody achieved in poor responders after revaccination may not persist for long periods. Although some data suggest that low levels of anti-HBs may protect against hepatitis B virus infection (8), further information is needed to establish the level and duration of protection after revaccination of nonresponders and nonresponders.

A correlation between HLA type and poor responsiveness to vaccination has been reported for influenza A virus; in that situation, genetically linked poor responsiveness was overcome by using a different antigen preparation (32). In the murine model of immunologic nonresponsiveness to HBsAg, nonresponsiveness can be overcome by altering the route of inoculation or the antigen preparation (33). Similar efforts are needed to identify defects causing nonresponsiveness to hepatitis B vaccine and to augment the humoral response to the present vaccine.

Acknowledgments: The authors thank Eloise Watkins, R.N., M.P.H., for her assistance; Dr. Arlene A. McLean, Merck Sharp & Dohme Research Laboratory, for her support; and Diane B. Crayton for help in manuscript preparation and for secretarial assistance.

Grant support: in part by grant #HL-29583 from the National Institutes of Health, and by a grant from the Department of Virus and Cell Biology Research, Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania.


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Melphalan May Be a More Potent Leukemogen than Cyclophosphamide

We have evaluated the relation between alkylating agents and leukemia disorders in 3363 1-year survivors of ovarian cancer who were treated in five randomized clinical trials and at two large medical centers. Overall, 28 patients developed acute nonlymphocytic leukemia (expected, 1.2) and 7 developed preleukemia. A 93-fold increased risk for acute nonlymphocytic leukemia was seen in 1794 women treated with chemotherapy, the incidence of leukemic disorders was 7.7/1000 women per year. Risk was highest 5 to 6 years after the first treatment and appeared to decrease thereafter. The use of radiation therapy did not affect risk. The 10-year cumulative risk (mean ± SE) of acquiring a leukemia disorder was 8.5% ± 1.6% after treatment with any alkylating agent, 11.2% ± 2.6% after treatment with melphalan, and 5.4% ± 3.2% after cyclophosphamide treatment. A dose-response relationship was apparent in 605 women receiving melphalan and suggested in 333 women receiving cyclophosphamide. Women receiving melphalan were two to three times as likely to develop leukemic disorders than were women receiving cyclophosphamide. These data indicate that choice of chemotherapeutic agent and drug dosage may influence significantly the risk for long-term adverse effects of cancer therapy.

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AN INCREASED RISK for acute nonlymphocytic leukemia and preleukemia has been documented in patients treated for several hematopoietic malignancies (1-8) and solid tumors (9-14), including ovarian cancer (15-18). Etologic studies in women treated for ovarian cancer are particularly informative because of the lack of an intrinsic predisposition for acute nonlymphocytic leukemia (15), and because each of the primary treatments for cancer plays a major role in the management of this disease. Therefore, to explore more fully the association between risk for leukemia and specific alkylating agents, and to evaluate the relationship between cumulative drug dose and the eventual development of leukemia, we evaluated a large cohort of women treated for ovarian cancer in five randomized trials and at two large medical centers.

Patients and Methods
We confined the present survey to women who had survived at least 1 year after the diagnosis of ovarian cancer, because a 50% mortality rate exists during the first year and treatment-related leukemic disorders generally do not develop until after the second year (16, 19). We began with 1032 1-year survivors from our previous cohort (Princess Margaret Hospital: 224 patients; M.D. Anderson Hospital: 222 patients; the Gynecologic Oncology Group: 586 patients), updated their follow-up data, and obtained more detailed treatment data (16). Twelve of these patients had leukemic disorders. We also evaluated the records of 2331 new female patients: 1441 from the Mayo Clinic and 890 from the M.D. Anderson Hospital. Each institution identified all women who were diagnosed or referred for treat-
