GENETIC PREDICTION OF NONRESPONSE TO HEPATITIS B VACCINE

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Abstract In previous studies of the antibody response to hepatitis B vaccine in 598 subjects who received a full course of vaccination, we observed a bimodal response, with about 14 percent producing less than approximately 1000 radioimmunoassay (RIA) units. An analysis of the major histocompatibility complex (MHC) HLA and complement types of 20 of the subjects with the lowest responses indicated a greater-than-expected number of homozygotes for the extended or fixed MHC haplotype [HLA-B8,SC01,DR3]. This finding suggested that the lack of a normal response was a recessive MHC-linked trait.

In this study, we prospectively vaccinated five homozygotes and nine heterozygotes for this haplotype in the expectation that the homozygotes would produce much lower levels of antibody than the heterozygotes. When the antibody response was assessed two months after the third injection, four of the five homozygotes had produced very low levels (approximately 1000 units or less) of antibody (mean, 467 RIA units; range, <8 to 1266), whereas all nine heterozygotes produced more than 2500 RIA units (mean, 15,608; range, 2655 to 28,900) (P<0.01).

We conclude that the usual response to hepatitis B surface antigen is due to the presence of a dominant immune-response gene in the MHC and that a low response is due to the absence of such a gene and the presence on both chromosomes of MHC haplotypes (such as [HLA-B8,SC01,DR3]) that indicate such a response. (N Engl J Med 1989; 321:708-12.)

The ability to produce antibody in response to specific protein antigens has been shown to be controlled by autosomal dominantly expressed Class II genes of the major histocompatibility complex (MHC) in mice and other species. The demonstration of such genes in humans has been difficult. Although synthetic amino acid terpolymers have been very useful in delineating such genes in laboratory animals, applications to the analysis of the genetic determinants of the immune response in vitro have been difficult in humans and have yielded somewhat different results in different laboratories. The results have suggested the presence of dominant immune-response genes within the MHC, including the Class I region, but no associations have been found between the response or lack of response to these terpolymers in vitro and specific HLA alleles or haplotypes. Family studies of the histogenetic response of lymphocytes in vitro to purified group A streptococcal extracellular antigen have suggested that the response is controlled by dominantly expressed HLA-linked immune-response genes. The incidence of HLA-DR2 has been found to be increased among subjects allergic to the ragweed antigen Ra5. HLA-DR4 is associated with hyperresponsiveness to collagen in vitro, and this phenomenon appears to be due to the absence of specific suppressor T cells. Matsushita and coworkers have reported that low responses to pollen, schizophrenia antigen, and streptococcal cell-wall antigen are all associated with dominantly expressed immune suppressor genes. These and other studies have provided suggestive evidence that in humans, MHC determinants are critical to the induction or suppression of an immune response to a particular antigen, but chromosomal localization or identification of specific immune-response genes has not been achieved.

The recent development of an effective hepatitis B vaccine, consisting of hepatitis B surface antigen (HBsAg) purified from human plasma or produced by recombinant-DNA methods, was a major advance in preventing hepatitis B infection. However, vaccine failures commonly occur that cannot be explained by such variables as improper storage or administration of the vaccine or characteristics of the recipients, such as advanced age, obesity, or renal failure.

In a trial of hepatitis B vaccine in 598 health care workers who were initially seronegative, 4.1 percent produced less than 36 estimated radioimmunoassay (RIA) units of antibody to HBsAg (anti-HBsAg). The peak antibody response in the entire population of vaccinated subjects, which occurred two to
three months after a third injection, is shown in Figure 1. The distribution of peak antibody levels appears to be bimodal, with the lower peak including levels ranging from less than 8 to approximately 1000 RIA units. The overall median level was 7200, and the mean was 26,718 RIA units. This suggests that there are two populations of normal persons: a majority with a high antibody response and a minority of approximately 14 percent with a lower- to- undetectable response (hyporesponders and non- responders, including the 4.1 percent with < 36 RIA units of anti-HBsAg).

To evaluate the role of the MHC in this variation in antibody response, we attempted to identify persons homozygous for extended haplotypes among 20 persons with a very low antibody response to the vaccine (< 36 RIA units per milliliter) by determining HLA and complement types.14 Extended haplotypes are relatively fixed segments of DNA defined by specific combinations of HLA-B, B, C2, C4A, C4B, and DR markers.15 There are at least a dozen common extended haplotypes among white persons, of which the most common is [HLA-B8, SC01, DR3]. SC01 is a four-gene complement haplotype, or clonotype, expressed as B*53, C2*3, C4A*Q0, C4B*1, in which Q0 denotes a null gene. This analysis suggested an increased frequency of homozygotes for [HLA-B8, SC01, DR3].14 Homozygotes for this haplotype constitute approximately 0.9 percent of the normal white population.15 In the group with the very low response, we found two apparent homozygotes for [HLA-B8, SC01, DR3], in contrast to the expected number of 4.5 among the combined population of white nonresponders and normal responders. There was no statistically significant difference between the number of homozygotes for [HLA-B8, SC01, DR3] observed and that predicted from the Hardy-Weinberg distribution and the recessive inheritance of the lack of immune response. Most of the expected homozygotes for [HLA-B8, SC01, DR3] in the total vaccinated population were among the nonresponders. There was also a single homozygote for a less common extended haplotype, [HLA-B44, FC31, DR7], for which none were expected. There was one heterozygote for the two haplotypes, in contrast to the four expected.

The best candidates for human immune-response genes — the Class II MHC genes HLA-DR, DQ, and DP — have been found to be fixed on all independent examples of [HLA-B8, SC01, DR3] tested to date and generally differ from those on other extended haplotypes. For [HLA-B8, SC01, DR3], these genes are HLA-DR3a/w52a, DQw2a,16,17 and usually DPw1.18 Because the nonresponders included homozygotes for this haplotype, who thus had two identical sets of Class II molecules, we reasoned that a low response to the hepatitis B vaccine must be a recessive trait, and that a single responding haplotype inherited as a dominant trait is sufficient for a normal antibody response in humans, as in mice.19

Figure 1. Antibody Response in 598 Health Care Workers after a Standard Course of Intramuscular Immunization with Hepatitis B Vaccine.14

Because we believe that the DNA of MHC extended haplotypes is relatively fixed, at least in the regions of HLA-B, DR, and DQ,16,20,21 the most likely site for human immune-response genes, we have postulated that most, if not all, independent examples of [HLA-B8, SC01, DR3] in the general population of whites will lack the immune-response gene for HBsAg. We therefore reasoned that persons chosen at random who are homozygous for [HLA-B8, SC01, DR3], if vaccinated with HBsAg, will not make normal amounts of anti-HBsAg, whereas heterozygotes for [HLA-B8, SC01, DR3] will usually carry a response gene on their other chromosome and thus will have a normal antibody response.

METHODS

All 14 subjects whose MHC phenotype was known were vaccinated with three 20-μg doses of plasma-derived hepatitis B vaccine (Hepavax-B; Merck Sharp & Dohme, West Point, Pa.) intramuscularly in the deltoid region and with a single intramuscular booster injection of tetanus toxoid (Massachusetts State Laboratories, Jamaica Plain, Mass.). The second and third injections were given one and six months afterward. Antibody levels (expressed as estimated RIA units14) were determined by radioimmunoassay (AUSAB, Abbott Laboratories, North Chicago) two weeks and two months after the last vaccine injection. Anti-tetanus toxoid titers were determined by hemagglutination inhibition22 six weeks after the booster injection. The subjects were chosen because they and their family members had previously been studied for MHC haplotypes and they were heterozygous or homozygous for the extended haplotype [HLA-B8, SC01, DR3]. All but four subjects were healthy; Subject 1 had Type 1 diabetes mellitus and gluten-sensitive enteropathy, and Subjects 2, 6, and 10 had gluten-sensitive enteropathy. All subjects were white, none were over 52 years of age or obese, and none had renal failure. The subjects came from four families and were related as follows: Subjects 1 and 2 were siblings and were the children of Subjects 6 and 7; Subjects 4, 5, and 12 were siblings; Subject 3 was the child of Subjects 10 and 13 and the sibling of Subjects 11 and 14; and Subject 9 was
the offspring of Subject 8. Among the heterozygotes for [HLA-B8,SC01,DR3], Subjects 9, 11, 12, and 13 were also heterozygous for [HLA-B44,FC31,DR7]. HLA-B, HLA-DR, and complement Bf, C2, and C4 types were determined in the subjects and their family members (to assign haplotypes) according to standard methods. The antibody levels and the assignment of haplotypes were performed independently by separate technicians.

RESULTS

Table 1 shows the results of the study. The antibody levels in the homozygotes for [HLA-B8,SC01,DR3] ranged from less than 8 to 1266 RIA units per milliliter of serum two months after the third injection, with a mean of 467 RIA units. In contrast, the antibody levels in the heterozygotes for this haplotype ranged from 2655 to 28,900 RIA units per milliliter, with a mean of 15,608, so that there was no overlap of values between the two groups. For comparison, the overall mean response in the study population of 598 subjects was 26,718 RIA units. When we used the t-test to compare the log10 of the antibody levels in the homozygotes for [HLA-B8,SC01,DR3] with those in the heterozygotes, the difference had a high level of significance (P<0.005). Similarly, according to the Mann–Whitney test, the antibody levels in the homozygotes were significantly lower than in the heterozygotes (P<0.01). All subjects had normal titers of antibody to tetanus toxoid on testing or after booster vaccination, indicating the specificity of the reduced anti-HBsAg response; there was no difference in these levels between homozygotes for [HLA-B8,SC01,DR3] and heterozygotes.

There was a suggestive concordance between homozygous siblings of very low levels of anti-HBsAg (Subjects 1 and 2) and of the levels nearer the lower limit of normal (Subjects 4 and 5). There were no HLA-identical siblings among the heterozygotes.

Table 1. Response to Vaccination with HBSAg and Tetanus Toxoid in Haplotypes and Heterozygotes for the Extended HLA Haplotype [HLA-B8,SC01,DR3].*  

<table>
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<tr>
<th>SUBJECT NO.</th>
<th>AGE</th>
<th>SERUM ANTI-HBSAG AFTER INJECTION</th>
<th>ANTI-TETANUS TOXOID TITER</th>
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<tr>
<td></td>
<td>yr</td>
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<td>6 WK</td>
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</table>

*ND denotes not done.

The double heterozygotes for [HLA-B8,SC01,DR3] and [HLA-B44,FC31,DR7] had a mean anti-HBsAg response of 16,400 RIA units (range, 3200 to 28,900), which was no different from that of the responders overall or of [HLA-B8,SC01,DR3] heterozygotes with other haplotypes.

DISCUSSION

These results support our hypothesis that the production of anti-HBsAg is a dominant trait and that the inability to produce high titers of anti-HBsAg after adequate immunization is a recessive trait. Thus, the lack of response is due to an absence of immune response (T-cell help) rather than to the presence of immune suppression (T-cell suppression or induction of suppression). Our findings also support our hypothesis that most persons with independent examples of [HLA-B8,SC01,DR3] do not carry an immune-response gene for HBsA. A formal demonstration of the mode of inheritance of responsiveness and unresponsiveness to HBsAg will require studies in families. Although current data are incomplete, the findings that two pairs of homozygous (clearly HLA-identical) siblings were among the nonresponders and that their level of response may have been concordant are consistent with our hypothesis. If the differences in the level of response among nonresponding homozygotes for [HLA-B8,SC01,DR3] are real, they may be due to minor sequence differences in Class II regions in independent examples of the extended haplotype or to genes at other nonlinked loci that contribute to the immune response. Proof of the mechanism of hyporesponsiveness will require a direct examination of the parts played by antigen-presenting cells and helper and suppressor T lymphocytes as well as by B lymphocytes in the poor response of our subjects who were homozygous for [HLA-B8,SC01,DR3].

One of the heterozygotes (Subject 10) had a response at the lower end of the normal range. This person's other haplotype was not an extended haplotype but may have carried the gene for hyporesponsiveness. If our hypothesis is correct, a frequency of 0.14 for hyporesponders implies a hyporesponse-gene frequency in the general population of about 0.37. Ignoring the very real but complicating possibility of gene complementation in the human immune response (the cooperation of parts of two specific nonresponder haplotypes that results in a response), one would expect three nonresponders among the nine heterozygotes for [HLA-B8,SC01,DR3]. Thus, our finding of one possible poor responder among the heterozygotes also fits the hypothesis.

Our findings in general are very similar to those observed in mice. Congenic inbred strains of mice are homozygous at all loci, including the MHC. Strains with certain haplotypes, H-2d and H-2b, respond poorly to HBsAg, whereas mice with other MHC haplotypes respond with adequate or high levels of antibody. Mice produced by the first-generation cross (F1) are heterozygous for the parents' MHC haplo-
types and respond normally if one parent is a responder. Breeding experiments of this kind have confirmed the dominant expression of the responder MHC haplotype. Because of their relative fixity of DNA, extended haplotypes in humans are analogous to the MHC haplotypes of inbred strains of mice and are thus potent tools for exploring human immunologic functions.

Because we had found one apparent homozygote and three apparent heterozygotes for the extended haplotype [HLA-B44,FC31,DR7], one of whom was also heterozygous for [HLA-B8,SC01,DR3], among the 20 poor responders in our previous study, the antibody responses of the four double heterozygotes in the present study were of interest. All had normal responses, and the mean antibody level among them was the same as that among the other heterozygotes. We have not yet prospectively immunized homozygotes for [HLA-B44,FC31,DR7] to confirm that it is a nonresponder haplotype. If it is, complementation between [HLA-B8,SC01,DR3] and [HLA-B44,FC31,DR7] may be common, resulting in a normal response. Additional work needs to be done to resolve these questions.

Our results are different from those of Watanabe and coworkers, who found that the lack of response to HBSAg in Japanese subjects was apparently mediated by dominantly expressed, antigen-specific immune suppression and was associated with the haplotype HLA-Bw54,DR4,DRw53, common in the Japanese population. They also found that nonresponders were largely heterozygous for the MHC markers, and therefore reasoned that the postulated immune-suppression gene was dominantly expressed. The discrepancy between our results and those of Watanabe et al. and of Chiu and coworkers, who also found evidence of suppression in nonresponders, could be due to ethnic differences in the mechanism of nonresponsiveness to HBSAg. The 22 percent rate of nonresponsiveness in the first Japanese study was surprisingly high, but it could also have been based on ethnic factors or related to the route of immunization (subcutaneous injection).

The haplotype [HLA-B8,SC01,DR3] is the most extended haplotype among white persons of European descent and is associated with a wide variety of diseases with autoimmune features in this population, including Type I diabetes mellitus, gluten-sensitive enteropathy, and probably many others. There is evidence that bearers of this haplotype have defects in the clearance of IgG-coated red cells, hyperresponsivity on mixed-lymphocyte culture, decreased proliferative response of lymphocytes to mitogens, increased response to wheat antigen in vitro, and decreased suppression of immunoglobulin synthesis in response to concanavalin A. Furthermore, chronic active hepatitis is associated with HLA-B8 and DR3. All these studies have examined only phenomena in heterozygotes for the haplotype. Nevertheless, the results suggest that different immunologic functions encoded by genes on [HLA-B8,SC01,DR3] may be responsible for susceptibility to different autoimmune diseases, rather than a single global abnormality resulting in autoimmunity in general. Certainly, the hyperresponsiveness characteristic of many autoimmune diseases contrasts with the hyporesponsiveness to HBSAg that we found.

We are indebted to Maureen Oliver for her help in obtaining blood samples; to Merck Sharp & Dohme and Dr. Dennis Economy for the generous donation of Heptavax-B for these studies; to Dr. Eric Lander for help with data analysis; to the CBR Laboratories and Dr. David Bing for the quantification of anti-HBSAg; and to the Boston Inter-Hospital Hepatitis B Vaccine Study Group for providing the data on the anti-HBSAg response of the 598 health care workers. The study group is composed of the following members: Jules L. Dienstag, M.D. (Massachusetts General Hospital), B. Frank Polk, M.D. (Brigham and Women's Hospital), David R. Snyderman, M.D. (New England Medical Center), Donald E. Craven, M.D. (Boston City Hospital—University Hospital), Richard Platt, M.D. (New England Deaconess Hospital), Clyde S. Cruess, M.D. (Beth Israel Hospital), Barbara G. Werner, Ph.D., and George F. Grady, M.D. (State Laboratory Institute, Department of Public Health, Commonwealth of Massachusetts).

REFERENCES
DECREASED INCIDENCE OF VENTRICULAR LATE POTENTIALS AFTER SUCCESSFUL THROMBOLYTIC THERAPY FOR ACUTE MYOCARDIAL INFARCTION

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Abstract  In some patients with acute myocardial infarction, low-amplitude potentials that prolong the QRS complex, termed “late potentials,” can be recorded on a signal-averaged electrocardiogram. The presence of these late potentials is known to be associated with an increase in the risk of ventricular tachycardia and sudden death. Because patients with acute myocardial infarction who receive thrombolytic therapy have a reduced incidence of ventricular tachyarrhythmia and sudden death, we sought to determine whether such patients also have a decreased incidence of late potentials.

We studied 106 patients less than 75 years of age who were admitted with a first myocardial infarction and in whom a signal-averaged electrocardiogram was recorded within 48 hours of admission. Within four hours of the onset of chest pain, tissue plasminogen activator (t-PA) was given to 44 patients, and 62 were treated conventionally.

VENTRICULAR late potentials are bursts of low-amplitude potentials that prolong the QRS complex on a signal-averaged surface electrocardiogram. Their presence in patients with acute myocardial infarction has recently been described as a marker for the propensity to have both spontaneous and in-

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In the t-PA group, late potentials were recorded in 2 of 44 patients (5 percent), as compared with 14 of 62 (23 percent) in the conventionally treated group (P = 0.01). Furthermore, among the patients treated with t-PA, continued occlusion of the infarct-related artery was related to the presence of late potentials. In the t-PA group, late potentials were recorded within 24 hours of angiography in 2 of the 6 patients with an occluded infarct-related artery, as compared with none of the 38 patients with a patent infarct-related artery.

Our data suggest that successful thrombolytic therapy is associated with a marked reduction in the incidence of late potentials on the signal-averaged electrocardiogram. Long-term follow-up will be required to determine whether this finding predicts a decreased incidence of subsequent ventricular tachyarrhythmia and sudden death. (N Engl J Med 1989; 321:712-6.)

ducible ventricular tachyarrhythmia, as well as a predictor of sudden cardiac death.17 These potentials have been demonstrated to be related to slow and inhomogeneous conduction within damaged cardiac tissue.18,19 Thrombolytic therapy has been shown to reduce mortality after acute myocardial infarction15-17, although this effect appears to be related primarily to a decrease in infarct size after early reperfusion, the lower mortality rate may also reflect improved ventricular electrical stability.18 Indeed, clinical studies of