The Immune Response to Hepatitis B Vaccine in Humans: Inheritance Patterns in Families

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Summary

We have recently shown that the human antibody response to the hepatitis B virus surface antigen (HBsAg) vaccine is major histocompatibility complex (MHC) associated. In studies of nonresponders to the vaccine, we found an increased incidence of individuals homozygous for human histocompatibility leukocyte antigen (HLA) proteins associated with the extended (conserved) haplotype [HLA-B8,SC01,DR3]. In later prospective vaccination trials, we showed that none of five individuals homozygous for this haplotype developed more than 1,300 radioimmunoassay (RIA) units of antibody (mean, 467 RIA units), while all heterozygotes made at least 2,500 RIA units (mean antibody level, 15,608 units). Our results suggested that [HLA-B8,SC01,DR3] lacks an immune response gene for HBsAg, and that response is inherited in a dominant fashion. To provide further evidence for this hypothesis, we have now analyzed the results of HBsAg immunization in families. 43 members of 10 families were immunized with the hepatitis B vaccine, including seven families where at least one member bore the haplotype [HLA-B8,SC01,DR3], and three families where one member had already received, but failed to respond to, the vaccine. In two of these three families, the presence of [HLA-B8,SC01,DR3] was subsequently found. Of nine MHC-identical sibling pairs in the study, both members of eight pairs had similar antibody responses (five nonresponder and three responder pairs). In all families with such sibling pairs, including the discordant pair, rank-ordering members by antibody level demonstrated that no relative's value came between the sibling pair values. Furthermore, of nine [HLA-B8,SC01,DR3]-haplotype-homozygous individuals, six were nonresponders, and two others had only low-normal responses. [HLA-B8,SC01,DR3]-heterozygous family members always had higher levels of antibody than their homozygous relatives. Linkage analysis of nonresponse to HLA haplotypes revealed a maximum likelihood LOD (logarithm of the odds) score of 6.3 at a recombination fraction of 0.1. The MHC association with lack of antibody response to HBsAg was not seen with tetanus immunization, where 1 of 20 HBsAg responders and 1 of 21 poor or nonresponders had tetanus titers of <1:512; both tetanus nonresponders were [HLA-B8,SC01,DR3] heterozygotes. Our results indicate that: (*a*) response to the HBsAg vaccine is MHC linked, and inherited in a dominant fashion; (*b*) an abnormal or missing immune response (Ir) gene for HBsAg is a characteristic of most examples of the extended haplotype [HLA-B8,SC01,DR3]; and (*c*) other haplotypes also have abnormal or missing Ir genes for HBsAg.

The immune response in mice to a variety of protein antigens is determined by class II genes of the MHC (1). The identification of relationships between murine H-2 gene complexes and the ability to recognize specific antigens has been facilitated by the use of inbred and H-2 congenic mouse strains, and their recombinant variations, in in vivo immunization experiments. For example, the response to the surface polypeptide antigen of the hepatitis B virus (HBsAg)[1] has been shown to be linked to MHC class II genes. Mice with the H-2^f and H-2^s haplotypes produce negligible titers of anti-HBs after immunization with the HBsAg vaccine (in

[1] *Abbreviations used in this paper:* HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; Ir, immune response.

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its current formulation, consisting of the S region protein), while other strains produce either intermediate (H-2^b, H-2^k) or high (H-2^d, H-2^q) levels (2). F_1 hybrids respond normally to the hepatitis vaccine provided that one parent is a responder (3). No evidence has been uncovered for HBsAg suppressor cells in nonresponder strains (4). These experiments have established the presence, in responder mice, and absence in nonresponders, of one or more dominant immune response (Ir) genes related to formation of antibody to HBsAg, although their precise location within the MHC remains unknown.

The murine and human MHC share many common features. However, identification of specific MHC genes in humans related to immune response has been considerably more difficult, because human populations are largely outbred, and carry diverse HLA antigens reflecting the marked polymorphism of this gene system. Despite these impediments, we have recently demonstrated that the human antibody response to immunization with the HBsAg vaccine is MHC associated. Many clinical studies have demonstrated that up to 7.5% of otherwise healthy HBsAg vaccine recipients are poor or nonresponders (5–11). In our initial study of a group of such nonresponders, we noted an increased incidence of individuals presumably homozygous (by phenotype) for the HLA class I gene HLA-B8, the class II gene HLA-DR3, and the genes for the complement proteins *BF*S*, *C2*C*, *C4A*Q0* (a null gene), and *C4B*1* (12). These MHC alleles are commonly inherited en bloc as an extended (conserved) haplotype, abbreviated [HLA-B8,SC01,DR3]. We and others have shown that in unrelated individuals with MHC markers for a number of different extended haplotypes, the genetic material along the entire area of the chromosome between HLA-B and HLA-DR/DQ is relatively fixed (13–16); thus, extended haplotypes may be considered similar to the H-2 haplotypes of inbred mice.

To test in vivo the hypothesis that the extended haplotype [HLA-B8,SC01,DR3] might be associated with absence of an immune response gene for HBsAg, we immunized individuals bearing this haplotype. 2 mo after the final injection, none of the five haplotype-homozygous subjects had >1,300 RIA U of antibody (mean, 467 RIA U), while all of the heterozygotes produced >2,500 RIA U (mean, 15,608 U) (17). We concluded from these results that the extended haplotype [HLA-B8,SC01,DR3], similar to nonresponder mouse haplotypes, lacked an immune response gene for HBsAg. Furthermore, our results suggested that in the [HLA-B8, SC01,DR3] heterozygote responders, the immune response gene present on the other haplotype functioned in a dominant manner. To further characterize the inheritance pattern of the immune response to HBsAg in relation to the MHC, we now report the results of HBsAg immunization studies in families.

Materials and Methods

Subjects. Families of subjects recruited for the immunization trial were from two sources: families where at least one individual bore the haplotype [HLA-B8,SC01,DR3] (seven families), identified from a Center for Blood Research database of >10,000 MHC-typed individuals, and families where an individual member had failed to respond to a standard immunization protocol with hepatitis B vaccine (three families). The results of hepatitis immunization in some members of four families, who were either homozygous or heterozygous for the haplotype [HLA-B8,SC01,DR3], have been previously reported (17).

Vaccines and Antibody Determinations. Hepatitis B vaccine, initially plasma derived and later of recombinant origin, was kindly supplied by Merck, Sharp & Dohme (Lancaster, PA). The immunogenicity of the two sources of vaccine is identical (18); however, for uniformity, all individuals in a family received either one or the other source of vaccine consistently. A standard vaccination protocol was used: 20 μg of vaccine was administered intramuscularly into the deltoid at the start of immunization, and again 1 and 6 mo after the first dose. Antibody to HBsAg was determined by RIA (AUSAB; Abbott Laboratories, North Chicago, IL) and expressed as estimated RIA U. Antibody was measured before immunization and 2 mo after the last vaccine injection. Antibody to HBc was determined by a competitive inhibition enzyme immunoassay (Corzyme; Abbott Laboratories). Individuals who had not received tetanus toxoid booster in the last 10 yr were offered reimmunization with tetanus toxoid. Anti–tetanus toxoid titers were determined by hemagglutination inhibition.

Histocompatibility Studies. MHC markers were determined in previously untested individuals as follows. Blood samples for complement typing were anticoagulated with EDTA, and the plasma and red cells were separated and frozen at −80°C until thawed for analysis. For HLA class I and II markers, heparinized blood samples were diluted with equal volumes of RPMI 1640, after which lymphocytes were separated by Ficoll-Hypaque, frozen, and stored in vapor-phase liquid nitrogen until thawed for analysis. Complement types were determined by agarose gel electrophoresis for BF and C4, and isoelectric focusing for C2, as described previously (19–21). Complement results are reported as the abbreviated compilation of the alleles BF, C2, C4A, and C4B, in that arbitrary order. Serotyping for HLA-A, -B, -C, -DR, and -DQ was performed using standard National Institutes of Health microcytotoxicity techniques.

Statistics. Statistical significance was determined using Fisher's exact test. Maximum likelihood statistical analysis (logarithm of the odds [LOD] scoring) was used to test for linkage between nonresponse to the hepatitis B vaccine and HLA haplotypes (22). For the purposes of this analysis, nonresponse was considered recessive, and the offspring from two-generation families with the extended haplotype [HLA-B8,SC01,DR3] were scored as phase known with nonresponse linked to that haplotype.

Results

Study Enrollment. 43 members of 10 families were immunized with the hepatitis B vaccine. Families A–E each included at least one member homozygous for the haplotype [HLA-B8,SC01,DR3]. Families F and G had members who were heterozygous for this haplotype, but no homozygous individuals. Families H, I, and J each included a known vaccine nonresponder. The proband nonresponder in family J, an emergency medicine technician, contracted hepatitis B 6 mo after the failed immunization attempt. He had a typical course for acute infection, and made an uneventful recovery, with the disappearance from his serum of the surface antigen, HBsAg, and the appearance of antibody to the nucleocapsid

antigen, HBcore; however, antibodies to HBsAg were never detected.

Antibody Levels. No antibodies to HBsAg were detected in any subject before immunization. Antibody levels 2 mo after the last vaccine injection are shown in relation to each family's pedigree and HLA type (Fig. 1). Our previous study revealed a bimodal distribution of antibody response in normal vaccine recipients, with some overlap between nonresponder and responder populations at antibody levels between 800 and 2,000 RIA U (17). Based on these data, subjects in the current study were considered nonresponders if antibody levels were <2,000 RIA U, and normal responders if antibody levels were ≥2,000 RIA U.

Antibody Levels in HLA-identical Siblings. If antibody response is linked to the MHC, antibody levels in HLA-identical siblings should be similar or identical. Fig. 2 depicts antibody response in the 43 subjects, and highlights nine pairs of HLA-identical siblings from eight families (including two from family H). In eight of nine pairs, both members made similar levels of antibody in response to immunization: in five pairs, both individuals were nonresponders (families A,C,I,J, and one of the two pairs in H), and in three pairs, both were responders (families B,D, and G). Family H contained the only discordant pair, consisting of one individual who was a nonresponder, and one with an exuberant antibody response. Even including the discordant family H pair,

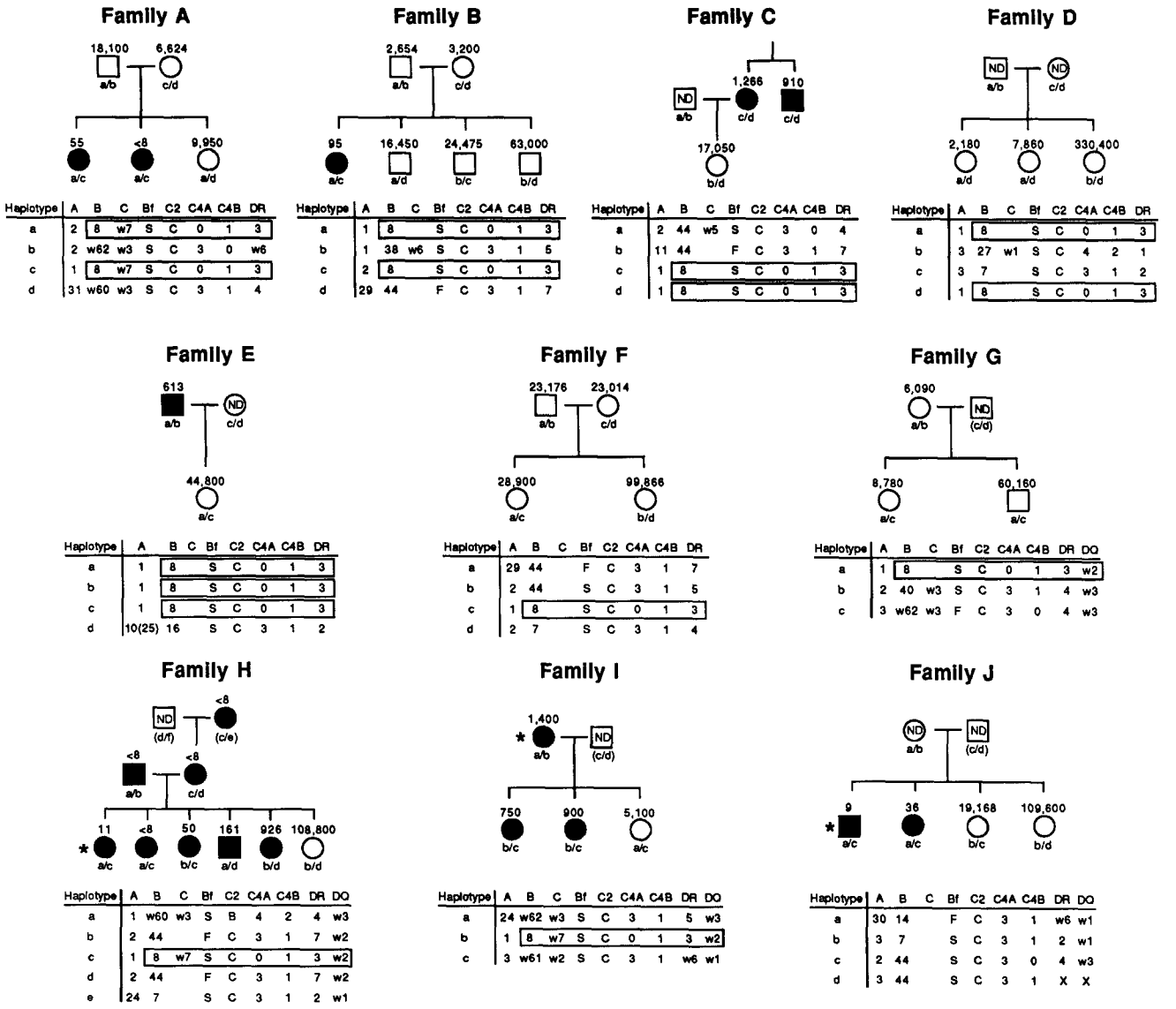

Figure 1. Family pedigrees. HLA markers found on each haplotype are listed in the table below the pedigree. The extended haplotype [HLA-B8,SC01,DR3] is outlined. The number over each family member is the level of antibody to HBsAg 2 mo after the third injection. (○) Members with normal antibody response (≥2,000 RIA U); (●) nonresponders (<2,000 RIA U). ND, not vaccinated. *Proband nonresponder.

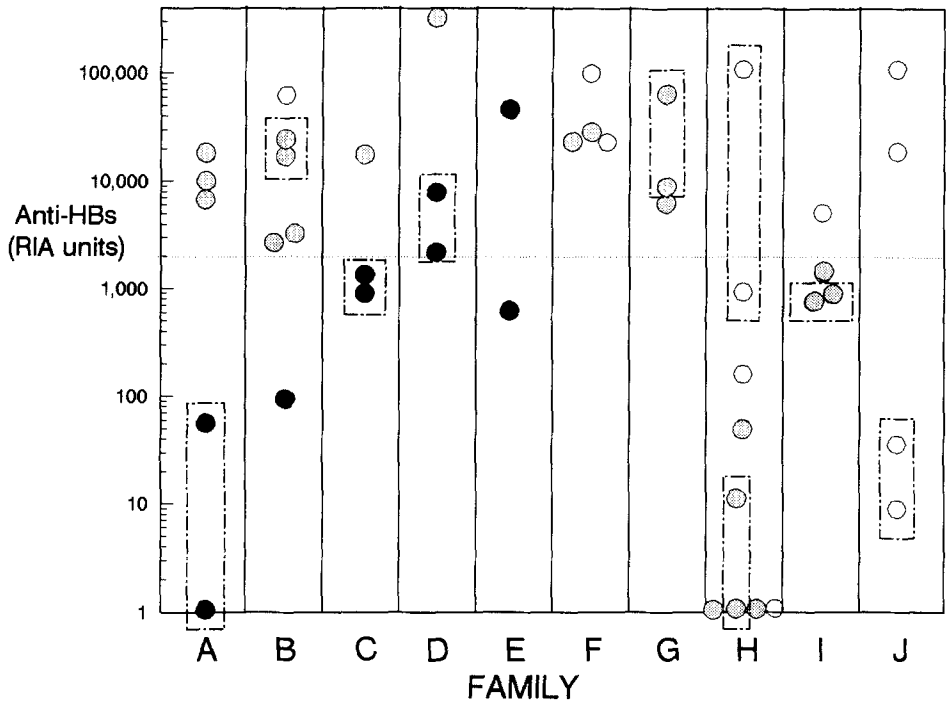

Figure 2. Antibody response versus presence of [HLA-B8,SC01, DR3]. (●) [HLA-B8,SC01,DR3] homozygous; (⊜) [HLA-B8,SC01, DR3] heterozygous; (○) other haplotypes. Boxed points are HLA-identical sib-pairs.

it can be seen in Fig. 2 that the HLA-identical sibling pairs always occur sequentially in rank order by antibody level, without intervening haplo- or nonidentical relatives. Analysis of concordance was carried out using six of the seven families with a nonresponder member (Table 1, families A,B,C,E,I, and J; family H, with eight nonresponders, was excluded because it was not informative). In each of these six families, a vaccine nonresponder (when possible, one member of an HLA-identical pair) was compared against all other family members. This analysis revealed that all HLA-identical siblings were concordant (nonresponders), and 13 of 14 haplo- or nonidentical siblings were discordant (antibody responders) ($p < 0.002$ by Fisher's exact test).

Antibody Levels in [HLA-B8,SC01,DR3] Haplotype-homozygous Individuals. If nonresponse to the hepatitis B vaccine is associated with the [HLA-B8,SC01,DR3] extended haplotype, and is due to the absence of an Ir gene on this haplotype, haplotype-homozygous individuals should in general be nonresponders, and individuals heterozygous for this haplotype should be responders, provided that the other haplotype contains a normally functioning Ir gene. This hypothesis is supported by the results shown in Fig. 2, where antibody response in relation to the HLA extended haplotype [HLA-B8,SC01,DR3] is shown. In families A, B, and C, five extended-haplotype-homozygous individuals (previously reported) were all antibody nonresponders (17), while eight heterozygotes made normal levels of antibody. Two [HLA-B8,SC01,DR3]-homozygous siblings in family D did produce antibody; however, the levels of antibody produced were at the low end of the normal range (2,180 and 7,860 RIA U), and distinct from the response of their haplotype-heterozygous sibling (330,400 RIA U). Family E contained one [HLA-B8,SC01,DR3] haplotype-homozygous nonresponder, but also the only [HLA-B8,SC01,DR3] homozygous individual, out of the nine homozygous study subjects in five families, who responded vigorously to the hepatitis vaccine (44,800 RIA U).

Table 1. *Concordance of Antibody Response and HLA Type*

Family	HLA type	Concordant	Discordant
A	Identical	1	0
	Nonidentical	0	3
B	Identical	0	0
	Nonidentical	0	5
C	Identical	1	0
	Nonidentical	0	1
E	Identical	0	0
	Nonidentical	0	1
I	Identical	1	0
	Nonidentical	1	1
J	Identical	1	0
	Nonidentical	0	2
Total	Identical	4	0
	Nonidentical	1	13

In six families with a vaccine nonresponder, each additional family member is categorized by comparison with the nonresponder based on HLA haplotype and antibody response.

Two families, F and G, contained individuals heterozygous for the [HLA-B8,SC01,DR3] haplotype, but no homozygotes. All members of families F and G (seven individuals, including five heterozygotes for the haplotype) made normal levels of antibody to hepatitis B surface antigen. In these seven families (A–G), six of nine haplotype homozygotes were nonresponders, versus none of 14 heterozygotes, a highly significant difference ($p < 0.002$).

Families H and I, chosen because each contained a known nonresponder, were found to include members with the haplotype [HLA-B8,SC01,DR3]. Only in these families, where the proband nonresponders were [HLA-B8,SC01,DR3] heterozygous, were six other heterozygotes for the haplotype also nonresponders.

Linkage Analysis. Sequential LOD scores were obtained for families A–E, I, and J. Three uninformative families were excluded from analysis: families F and G because they contained no nonresponder members, and family H because both parents and one grandparent were nonresponders. In the remaining families, the highest positive LOD score, 6.3, occurred at a recombination fraction of 0.10.

Antibody Levels after Tetanus Toxoid Immunization. To demonstrate that the MHC association with lack of antibody response to hepatitis B immunization is distinct from the lack of response to other vaccines, we studied the antibody response to tetanus toxoid in our subjects (Table 2). Of 43 subjects, two had low initial antibody titers (1:16) but did not undergo booster immunization with tetanus toxoid; the other 41 individuals either had levels of antibody to tetanus that were ≥ 1:512 at the start of the study, or else agreed to booster immunization with tetanus toxoid. Of these 41 subjects, 18 were nonresponders to HBsAg; however only two of 41 had poor responses to tetanus toxoid. Of these two, one (a member of family A) had a normal antibody level after hepatitis B immunization, but a tetanus titer of 1:16; the other (the oldest member of family H) was a hepatitis nonresponder whose tetanus titer went from 1:16 to 1:256 after vaccination. Both subjects were heterozygous for the HLA haplotype [B8, SC01,DR3].

Evidence for Natural Infection with HBV in Study Subjects. Exposure to the natural hepatitis B virus through infection (as opposed to the purified S protein) was able to circumvent nonresponsiveness in one case report (23). We therefore looked for evidence of natural infection with hepatitis B virus (HBV), by determining the presence of antibodies to HBc, in three subjects (and their families) where nonresponsiveness was predicted, but a better-than-expected or normal antibody response was seen: members of family D, where two HLA-identical [HLA-B8,SC01,DR3] homozygous siblings made low normal amounts of antibody; family H, where an HLA-identical sibling of a nonresponder made a normal amount of antibody; and family E, where an [HLA-B8,SC01,DR3] haplotype-homozygous individual made a normal amount of antibody. We also studied family J, where one individual was known to have been infected with HBV. Only the proband nonresponder of family J had a high titer of anti-HBc (positive at a dilution of (1:100), consistent with his history of previous infection with HBV; all other serum samples in this and the other three families were negative.

Discussion

Our results in this human study of immune response to the hepatitis B vaccine parallel those previously reported in murine models using the S protein. First, we have shown that the response to hepatitis B vaccine in humans is MHC linked, as evidenced by the LOD score of 6.3 at a recombination fraction of 0.1. In five families (A,C,H,I, and J), nonresponders to the vaccine had an HLA-identical sibling, for a total of six pairs (two pairs in family H). In five of these six pairs, siblings made virtually identical levels of antibody. In only one pair, in family H, did one of the two [HLA-B44,FC31,DR7]-haplotype-homozygous individuals develop an exuberant level of antibody. This subject had no antibodies to hepatitis B core antigen, ruling out the possibility that she had been previously infected with HBV, and in this way had circumvented her sibling's nonresponsiveness to HBsAg. It is possible, instead, that unidentified non-MHC-linked factors that enhanced T cell function nonspecifically helped circumvent nonresponsiveness in this case. In mice, for example, nonresponders of the H-2^s haplotype can be induced to make antibody when 10 times the dose of antigen is used for immunization, or when an alternative injection site (footpad, as opposed to intraperitoneal, injection) is used (4). These results suggest that the B cell populations necessary for anti-HBs production are present in nonresponders, and that the absence of specific T cell help, while critical to nonresponder status, can be overcome. Alternatively, somatic changes in the MHC, undetected by our serologic tests for

Table 2. *HBsAg Versus Tetanus Toxoid Antibodies*

	Tetanus antibodies		
HBsAg response	<1:512 (no. of subjects)	>1:512 (no. of subjects)	Total
Normal (≥2,000 RIA U)	1	22	23
None (<2,000 RIA U)	1	17	18
Total	2	39	41

MHC proteins, but sufficient to change peptide binding ability or other characteristics of immune response genes, may have occurred in one but not the other sibling. The identification and location of such a change would provide key information about Ir genes and their function.

Second, we have provided further proof with this study that individuals homozygous for the extended haplotype [HLA-B8,SC01,DR3] are nonresponders to hepatitis B immunization, but normal antibody responders to tetanus toxoid. Nine individuals from five different families (A–E) were homozygous for this haplotype. All nine responded normally to tetanus toxoid; but six were HBsAg nonresponders, and two had only low normal responses. Only one individual, in family E, was an exception in that she made a normal level of antibody to HBsAg (44,800 RIA U). In this family, three separate examples of [HLA-B8,SC01,DR3] were present: the father was a haplotype-homozygous nonresponder, and the mother, who refused vaccination, was [HLA-B8,SC01,DR3] heterozygous. The daughter inherited both the maternal and one of the two paternal [HLA-B8,SC01,DR3] haplotypes. She had no detectable antibodies to HBcAg. The presence of anti-HBs in this subject suggests that the maternal [HLA-B8,SC01,DR3] haplotype, although grossly identical with the paternal and other examples of this extended haplotype, may differ in fine structure in the region of the immune response gene.

In families with [HLA-B8,SC01,DR3] homozygotes (families A–D), [HLA-B8,SC01,DR3] heterozygotes and nonidentical family members all had better antibody responses than their [HLA-B8,SC01,DR3]-haplotype-homozygous relatives: all 10 heterozygous or nonhaplotype-bearing subjects were antibody responders. These family studies support the hypothesis that the haplotype [HLA-B8,SC01,DR3] lacks a normal immune response gene for HBsAg, and the immune defect associated with this haplotype can be overcome provided that an individual inherits a normal immune response gene on the other haplotype.

However, [HLA-B8,SC01,DR3] cannot be the only haplotype deficient in an Ir gene for HBsAg. Based on studies of antibody response in normal health care workers, and assuming an autosomal dominant mode of inheritance of Ir genes, we have hypothesized that the frequency of hyporesponse genes to HBsAg may approach a frequency of 0.37 (12, 17). Since [HLA-B8,SC01,DR3] has a frequency of 0.09 in Caucasians (13), its inheritance explains only 25% of nonresponder haplotypes to the vaccine in this population, and other haplotypes must be involved.

We therefore studied families H, I, and J, ascertained because a member was known to be a nonresponder to the hepatitis vaccine. Consistent with our expectations, the extended haplotype [HLA-B8,SC01,DR3] turned out to be present in two of the three families (H and I). In each case, the initial (proband) nonresponder was heterozygous for [HLA-B8, SC01,DR3] and another haplotype, suggesting that the second haplotype was also abnormal with regard to Ir gene(s) for HBsAg. In family H, the [HLA-B8,SC01,DR3]-heterozygous proband had an HLA-identical sibling who was also a nonresponder. In addition to these two family members, six other individuals, spanning three generations, were nonresponders, suggesting that all five haplotypes in this family (two examples of [HLA-B44,FC31,DR7], as well as [HLA-B8,SC01, DR3], [HLA-B7,SC31,DR2], and [HLA-Bw60,SB42,DR4]) were associated with nonresponse to the vaccine. The proband nonresponder in family I, also [HLA-B8,SC01,DR3] heterozygous, had no HLA-identical siblings. However, her daughters, both heterozygotes for the proband's [HLA-B8, SC01,DR3] haplotype, were also nonresponders. In this family, two additional haplotypes appeared to have defects in the Ir gene for HBsAg. Since [HLA-B8,SC01,DR3] lacks an Ir gene, the results in these two families support our hypothesis that haplotypes other than [HLA-B8,SC01,DR3] also lack an HBsAg Ir gene.

The results in family J are consistent with the hypothesis that this family carries two non-[HLA-B8,SC01,DR3]-bearing haplotypes (a and c), each with abnormal HBsAg Ir genes. The initial nonresponder (a/c haplotype) had an HLA-identical sibling, who also proved to be a nonresponder during the study. A sibling haploidentical with the nonresponders (b/c) made a normal amount of antibody (19,168 RIA U), and the nonidentical sibling (b/d) was an exuberant antibody producer (109,600 RIA U). An alternative explanation, that the a haplotype carries an immune suppression gene, cannot be ruled out in this family, because no a/d sibling existed for immunization studies. If the latter hypothesis were correct, however, family H would be the only one, of 10 in the study, where an immune suppression gene could be invoked.

Thus, our results suggest the following conclusions in humans regarding immunization to HBsAg: (*a*) response to the vaccine is MHC linked, and inherited in a dominant fashion; (*b*) an abnormal or missing Ir gene for HBsAg is a characteristic of most examples of the extended haplotype [HLA-B8,SC01,DR3], as evidenced by the lack of response to HBsAg immunization in nearly all individuals who are homozygous for this haplotype; and (*c*) other haplotypes also have abnormal or missing Ir genes for HBsAg.

Our findings, while similar to those in mice, differ from the interpretation of some Japanese workers of in vivo and in vitro data on nonresponding oriental subjects, which they report as consistent with the presence of an autosomal dominant immune suppression gene on a common Japanese haplotype [HLA-Bw54,DR4,DRw53] (24–26). However, some of these studies were based on an inferior mode of vaccine administration, the subcutaneous route, which resulted in a much higher incidence of nonresponders (22.4%) than found in other studies, and may have obscured interpretation of the data (25, 26). Our conclusions were based on results of a rigorous study protocol, which included the use of intramuscular injections in the deltoid muscle, and consistent use of antibody determinations 2 mo after the third vaccination. Furthermore, no Japanese family studies were done to prove that suppression was inherited as a dominant trait. Some groups have reported the presence of HBsAg-specific suppressor T cells in Japanese nonresponders (24, 25); however, we have been unable to demonstrate any effect of T cell suppression in Caucasian nonresponders using an in vitro proliferation assay (27).

A number of other possible associations between the [HLA-B8,SC01,DR3] haplotype, or markers for it, and a variety of diseases and in vitro immunologic abnormalities, have been reported over the years, including celiac disease (28, 29), dermatitis herpetiformis (30), diabetes mellitus (31, 32), alloimmunization to the platelet antigen PLA1 (33), rapidity of progression of HIV-related illness (34, 35), abnormal in vitro lymphocyte proliferation in response to PHA and other mitogens (36–39), and abnormal Fc receptor function (40). It is quite possible that this extended haplotype has many aberrant Ir and other genes. Our demonstration of a clear relationship between the extended haplotype [HLA-B8,SC01, DR3] and a defective Ir gene for HBsAg was facilitated by the study of individuals homozygous for this extended haplotype. In some of the other reports, where only heterozygotes were studied, or else discovered retrospectively, the influence of a wide assortment of second haplotypes, and the very high incidence of the [HLA-B8,SC01,DR3] haplotype in the northern European Caucasian population, make conclusions about MHC associations more tenuous. It is also not clear whether any of these additional immunologic abnormalities are related to the defective Ir gene for HBsAg on this haplotype. Since we found normal antibody responses to tetanus toxoid in all hepatitis B vaccine nonresponders who were haplotype homozygous for [HLA-B8,SC01,DR3], the lack of response to hepatitis vaccine is most likely antigen specific, rather than a more general problem with the immune response determined by this haplotype.

Neither the location of the Ir gene responsible for hepatitis B antibody production, nor the defect that results when it is missing or functioning poorly, is understood. However, the identification of [HLA-B8,SC01,DR3] and other haplotypes as MHC markers for nonresponse opens the way to more directed studies of HBsAg recognition and presentation in association with the MHC.

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References

1. Benacerraf, B. 1981. Role of MHC gene products in immune regulation. *Science (Wash. DC).* 212:1229.
2. Milich, D.R., and F.V. Chisari. 1982. Genetic regulation of the immune response to hepatitis B surface antigen (HBsAg). I. H-2 restriction of the murine humoral immune response to a and d determinants of HBsAg. *J. Immunol.* 129:320.
3. Milich, D.R., G.G. Leroux-Roels, and F.V. Chisari. 1983. Genetic regulation of the immune response to hepatitis B surface antigen (HBsAg). II. Quantitative characteristics of the humoral immune response to the a, d, and y determinants of HBsAg. *J. Immunol.* 130:1395.
4. Milich, D.R., H. Alexander, and F.V. Chisari. 1983. Genetic regulation of the immune response to hepatitis B surface antigen (HBsAg): III: Circumvention of nonresponsiveness in mice bearing HBsAg nonresponder haplotypes. *J. Immunol.* 130:1401.
5. Jilg, W., M. Schmidt, and F. Deinhardt. 1989. Four-year experience with a recombinant hepatitis B vaccine. *Infection.* 17:70.
6. Wainwright, R.B., B.J. McMahon, I.R. Bulkow, et al. 1989. Duration of immunogenicity and efficacy of hepatitis B vaccine in a Yupik Eskimo population. *J. Am. Med. Assoc.* 261:2362.
7. Stevens, C.E., and P.E. Taylor. 1986. Hepatitis B vaccine: issues, recommendations, and new developments. *Semin. Liver Dis.* 6:23.
8. Wismans, P., J. van Hattum, T. Stelling, J. Poel, and G.C. de Gast. 1988. Effect of supplementary vaccination in healthy non-responders to hepatitis B vaccination. *Hepato-gastroenterology.* 35:78.
9. Kramer, A., D. Herth, H.-J. von Keyserlingk, W.D. Ludwig, H. Hampl, D. Sommer, E.G. Hahn, and E.O. Riecken. 1988. Non-responsiveness to hepatitis-B vaccination: revaccination and immunogenetic typing. *Klin. Wochenschr.* 66:670.
10. Pasko, M.T., and T.R. Beam. 1990. Persistence of anti-HBs among health care personnel immunized with hepatitis B vaccine. *Am. J. Public Health.* 80:590.
11. Herbert, M., A.V.J. Butler, A.P.C.H. Roome, and E.O. Caul. 1989. Comparison of intradermal and intramuscular hepatitis B vaccination in university students. *Vaccine.* 7:395.
12. Craven, D.E., Z.L. Awdeh, L.M. Kunches, E.J. Yunis, J.L. Dienstag, B.G. Werner, B.F. Polk, D.R. Syndman, R. Platt, C.S. Crumpacker, G.F. Grady, and C.A. Alper. 1986. Nonresponsiveness to hepatitis B vaccine in health care workers: results of revaccination and genetic typings. *Ann. Int. Med.* 105:356.
13. Awdeh, Z.L., D. Raum, E.J. Yunis, and C.A. Alper. 1983. Extended HLA/complement allele haplotypes: evidence for T/t-like complex in man. *Proc. Natl. Acad. Sci. USA.* 80:259.
14. Alper, C.A. 1991. Major histocompatibility complex allele-

disease associations in the light of extended haplotypes. *In* The Immunogenetics of Autoimmune Diseases. N.R. Farid, editor. CRC Press, Boca Raton, FL. pg. 167.
15. Pollack, M.S., B. Keenan, F.T. Christiansen, T.J. Cobain, R.L. Dawkins, and G. Clayton. 1986. The immunological detection of a 21-OH deficiency mutation HLA supratype. *Am. J. Hum. Genet.* 38:688.
16. Zhang, W.J., M.A. Degli-Esposti, T.J. Cobain, P.U. Cameron, F.T. Christiansen, and R.L. Dawkins. 1990. Differences in gene copy number carried by different MHC ancestral haplotypes. Quantitation after physical separation of haplotypes by pulse field gel electrophoresis. *J. Exp. Med.* 171:2101.
17. Alper, C.A., M.S. Kruskall, D. Marcus-Bagley, D.E. Craven, A.J. Katz, S.J. Brink, J.L. Dienstag, Z. Awdeh, and E.J. Yunis. 1989. Genetic prediction of nonresponse to hepatitis B vaccine. *N. Engl. J. Med.* 321:708.
18. Brown, S.E., C. Stanley, C.R. Howard, A.J. Zuckerman, and M.W. Steward. 1986. Antibody responses to recombinant and plasma derived hepatitis B vaccines. *Br. Med. J.* 292:159.
19. Alper, C.A., T. Boenisch, and L. Watson. 1972. Genetic polymorphism in human glycine-rich beta-glycoprotein. *J. Exp. Med.* 135:68.
20. Alper, C.A. 1976. Inherited structural polymorphism in human *C2*: evidence for genetic linkage between *C2* and *Bf*. *J. Exp. Med.* 144:1111.
21. Awdeh, Z.L., and C.A. Alper. 1980. Inherited structural polymorphism of the fourth component of human complement. *Proc. Natl. Acad. Sci. USA.* 77:3576.
22. Morton, N.E. 1955. Sequential tests for the detection of linkage. *Am. J. Hum. Genet.* 7:277.
23. Bortolotti, F., C. Crivellaro, E. Pornaro, and G. Realdi. 1988. Hepatitis B in a nonresponder to hepatitis B vaccine. *Infection.* 16:119.
24. Chiou, S.-S., K. Yamauchi, T. Nakanishi, and H. Obata. 1988. Nature of immunological non-responsiveness to hepatitis B vaccine in healthy individuals. *Immunology.* 64:545.
25. Watanabe, H., M. Okumura, K. Hirayama, and T. Sasazuki. 1990. HLA-Bw54-DR4-DRw53-DQw4 haplotype controls nonresponsiveness to hepatitis-B surface antigen via CD8-positive suppressor T cells. *Tissue. Antigens.* 36:69.
26. Watanabe, H., S. Matsushita, N. Kamikawaji, K. Hirayama, M. Okumura, and T. Sasazuki. 1988. Immune suppression gene on HLA-Bw54-DR4-DRw53 haplotype controls nonresponsiveness in humans to hepatitis B surface antigen via $CD8^+$ suppressor T cells. *Hum. Immunol.* 22:9.
27. Egea, E., A. Iglesias, M. Salazar, C. Morimoto, M.S. Kruskall, Z. Awdeh, S.F. Schlossman, C.A. Alper, and E.J. Yunis. 1991. The cellular basis for lack of antibody response to hepatitis B vaccine in humans. *J. Exp. Med.* 173:531.
28. Alper, C.A., E. Fleischnick, Z. Awdeh, A.J. Katz, and E.J. Yunis. 1987. Extended major histocompatibility complex haplotypes in patients with gluten-sensitive enteropathy. *J. Clin. Invest.* 79:251.
29. Marsh, M.N., I. Bjarnason, J. Shaw, A. Ellis, R. Baker, and T.J. Peters. 1990. Studies of intestinal lymphoid tissue. XIV. HLA status, mucosal morphology, permeability, and epithelial lymphocyte populations in first degree relatives of patients with coeliac disease. *Gut.* 31:32.
30. Lawley, T.J., W. Strober, H. Yaoita, and S.I. Katz. 1980. Small intestinal biopsies and HLA types in dermatitis herpetiformis patients with granular and linear IgA skin deposits. *J. Invest. Dermatol.* 74:9.
31. Raum, D., Z. Awdeh, E.J. Yunis, C.A. Alper, and K.H. Gabbay. 1984. Extended major histocompatibility complex haplotypes in type I diabetes mellitus. *J. Clin. Invest.* 74:449.
32. Svejgaard, A., and L.P. Ryder. 1981. HLA genotype distribution and genetic models of insulin-dependent diabetes mellitus. *Ann. Hum. Genet.* 45:293.
33. Blanchette, V.S., L. Chen, Z.S. de Friedberg, V.A. Hogan, E. Trudel, and F. Decary. 1990. Alloimmunization to the PLA1 platelet antigen: results of a prospective study. *Br. J. Haematol.* 74:209.
34. Cameron, P.U., S.A. Mallal, M.A. French, and R.L. Dawkins. 1990. Major histocompatibility complex genes influence the outcome of HIV infection. Ancestral haplotypes with C4 null alleles explain diverse HLA associations. *Hum. Immunol.* 29:282.
35. Kaslow, R.A., R. Duquesnoy, M. VanRaden, L. Kingsley, M. Marrari, H. Friedman, S. Su, A.J. Saah, R. Detels, J. Phair, and C. Rinaldo. 1990. A1, Cw7, B8, DR3 HLA antigen combination associated with rapid decline of T-helper lymphocytes in HIV-1 infection: a report from the Multicenter AIDS Cohort Study. *Lancet.* 335:927.
36. Ambinder, J.N., N. Chiorazzi, A. Gibofsky, M. Fotino, and H.G. Kunkel. 1982. Special characteristics of cellular immune function in normal individuals of the HLA-DR3 type. *Clin. Immunol. Immunopathol.* 23:269.
37. Modica, M.A., G. Cammarata, and C. Caruso. 1990. HLA-B8,DR3 phenotype and lymphocyte responses to phytohemagglutinin. *J. Immunogenet.* 17:101.
38. Amer, A., G. Singh, C. Darke, and A.E. Dolby. 1986. Impaired lymphocyte responsiveness to phytohaemagglutinin associated with the possession of HLA-B8/DR3. *Tissue. Antigens.* 28:193.
39. McCombs, C., and J. Michalski. 1982. Lymphocyte abnormalities associated with HLA-B8 in healthy young adults. *J. Exp. Med.* 156:936.
40. Lawley, T.J., R.P. Hall, A.S. Fauci, S.I. Katz, M.I. Hamburger, and M.M. Frank. 1981. Defective Fc-receptor functions associated with the HLA-B8/DRw3 haplotype. *N. Engl. J. Med.* 304:185.