Response to Immunization with *Haemophilus influenzae* Type b Polysaccharide-Pertussis Vaccine and Risk of *Haemophilus* Meningitis in Children with the Km(1) Immunoglobulin Allotype

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Abstract. In experimental animals, immune responses to certain antigens are regulated by immunoglobulin allotype-linked genes. In an effort to detect such genes in humans, we examined the antibody responses of 74 healthy children with different Km(1) or Gm(23) allotypes to a *Haemophilus influenzae* type b vaccine (type b polysaccharide capsule-pertussis vaccine). The anticapsular antibody responses of black or white children with the Km(1) allotype were 4.6- to 9.5-fold higher than those of children who lacked this determinant ($P < 0.004$). No significant differences were found in antibody response with respect to the Gm(23) allotype. The frequencies of Km(1) and Gm(23) also were examined in 170 patients with *Haemophilus* meningitis, 71 patients with epiglottitis, and 173 control children. Km(1) was detected less frequently in black patients with meningitis (38%) than in those with epiglottitis (81%, $P < 0.002$) or in controls (66%, $P < 0.0007$). The relative risk of meningitis thus was 3.2-fold lower among black children with the Km(1) allotype than in those who lacked this allotype (odds ratio = 0.3, 95% confidence interval 0.2 to 0.6). However, the risk of meningitis was not decreased in white children with the Km(1) allotype (odds ratio = 1.0). There were no significant differences in the frequency of Gm(23) among the patient groups and controls. The Km(1) allotype but not the Gm(23) thus defines a subpopulation of children of both races who are high responders to this vaccine, and black children but not white children with the Km(1) allotype are at decreased risk of developing *Haemophilus* meningitis. These data indicate that in blacks, genes associated with Km(1) may affect immune response to a prototype type b *Haemophilus* vaccine, and perhaps interact with another factor related to race to affect susceptibility to *Haemophilus* meningitis.

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Introduction

Haemophilus influenzae type b is the most common cause of bacterial meningitis in children in North America (1). This organism also is an important cause of other serious infections such as acute epiglottitis. Socioeconomic status, environment, and racial factors have been found to affect the incidence of invasive *Haemophilus* disease in different populations (1–4). Recent evidence also suggests that genetic factors may influence susceptibility (2, 5–7).

Considerable evidence from studies of inbred strains of mice indicates that immune responsiveness is under genetic control (for examples, see references 8 and 9). A large number of immune response genes have been described in laboratory animals. The majority are linked to the major histocompatibility complex (8, 9). However, studies indicate that genes associated with immunoglobulin allotype determinants also may affect the antibody responses to certain antigens (10–13). Allotypes are hereditary antigenic determinants on Ig molecules found in some but not all individuals of a species (14). They are inherited in a Mendelian fashion and are codominant (i.e., both allelic genes are expressed in a heterozygote).

In humans, Gm allotype specificities are located on the constant region of the γ-heavy chain. Thus far, 25 Gm specificities have been detected. Three other allotype markers,

the Km factors, have been correlated with amino acid substitutions in the constant region of the κ-chain (14). The gene loci for Km and Gm specificities are not linked (they are located on different chromosomes). Two allotypes, Km(1) and Gm(23) (also designated as G2m [15]) (the latter, a specificity on IgG_2 subclass molecules), have been associated with altered immune responses to the capsular polysaccharide of *H. influenzae* type b (15, 16) and to other bacterial antigens (17, 18). Further, a recent preliminary report suggests that subjects with Gm(23) have a lower relative risk of developing invasive *Haemophilus* disease than those who lack this determinant (19).

The capsular polysaccharide of *H. influenzae* type b (a polymer of →3)-β-D-Ribf-(1 → 1)-ribitol-5-(phosphate→) (19a) is an important virulence determinant (20, 21). Nearly all invasive *Haemophilus* disease in children is caused by type b organisms (1–3), and organisms that have lost their capsule are no longer pathogenic in experimental animals (20, 21). Furthermore, antibody against the type b capsule confers protection against invasive *Haemophilus* disease (22–24). Preliminary data also suggest that this antibody is predominantly of the IgG_2 subclass (25) and is of restricted heterogeneity with respect to kappa/lambda light chain and clonatype distribution (26). It seemed possible that genes in linkage disequilibrium with those coding for Km(1) or Gm(23) allotypes might affect immunologic responses to the type b capsule, and thereby influence susceptibility to different manifestations of type b *Haemophilus* infection. This study, therefore, was designed to address the following questions: (*a*) Do genes associated with the Km(1) or Gm(23) allotypes affect immune responsiveness to an *H. influenzae* type b vaccine; (*b*) Do children with the Km(1) or Gm(23) allotypes have a different risk of developing *Haemophilus* meningitis or epiglottitis than children who lack these allotypes?

Methods

Comparison of the frequency of Km(1) or Gm(23) in patients and controls is described as follows.

Subject selection. Serum samples were obtained from 241 patients (87 blacks and 154 whites) with *H. influenzae* type b meningitis or epiglottitis confirmed by positive cultures of blood or cerebrospinal fluid, or by detection of capsular antigen in cerebrospinal fluid, serum, or urine by countercurrent immunoelectrophoresis. 170 patients had meningitis and 71 had epiglottitis. All of the patients resided in the greater St. Louis metropolitan area and had been hospitalized at either St. Louis Children's Hospital or Cardinal Glennon Memorial Hospital for Children. Serum samples also were obtained from 173 control children (83 blacks and 90 whites) without a history of type b *Haemophilus* disease. These subjects also resided in the greater St. Louis area and were volunteers in vaccine trials conducted by our group. The control children were recruited from several sources: a prepaid medical care group associated with Washington University, an unaffiliated family practice clinic in a local community hospital, and two private practices serving primarily families of low income. Patients and control individuals < 7 mo of age were excluded from this study because in this age group the presence of small amounts of maternal antibody can interfere with determination of Ig allotypes. The frequencies of different HLA A, B, and DR specificities in the white patients and controls have been reported (6).

Response to immunization. 56 of the control children from St. Louis, MO (21 blacks and 35 whites), and 18 additional white children from Danbury, CT were immunized. Serum samples from the children in Connecticut were kindly provided by Dr. Martha Lepow, Albany Medical College, Albany, NY. The children ranged in age from 9 to 30 mo (mean±SD = 18.6±5.7). 25 of the children from St. Louis served as controls in a previous study and their antibody responses have been reported (7). A 0.5-ml dose containing 10 μg of the type b capsular polysaccharide and four opacity units of pertussis vaccine (PRP-pertussis vaccine) was administered intramuscularly. The vaccine (lots *7-1374-016A, 026A,* and *039A*) was supplied by Lederle Laboratories (Division of American Cyanamid Co., Pearl River, NY). Characterization of the vaccine (27) and its immunogenicity in laboratory animals (27, 28) and humans (7, 29–32) have been reported. Blood samples were obtained by venipuncture before immunization and 4 to 6 wk later. Serum antibody concentrations against the type b capsular antigen were measured by a modified Farr radioantigen binding assay using ^{3}H-labeled type b polysaccharide antigen (33). The minimum amount of antibody detectable in this assay was 25 ng antibody protein per milliliter of serum, as determined by dilutions of *H. influenzae* type b human reference serum (SK) (34). The correlation coefficient between values obtained on serum samples assayed on different days was >0.90.

IgG and IgM anti-PRP antibody were measured by an enzyme-linked immunosorbent assay using type b capsule-tyramine as described (7). Alkaline phosphatase conjugated goat anti-human IgG or IgM (heavy chain specific, Sigma Chemical Co., St. Louis, MO) were used for Ig detection. Titers were determined graphically as the reciprocal of the serum dilution producing an absorbence of 0.3 as described. For IgG the assay was considered complete when wells containing a 1:5,000 dilution of a standard human serum containing 35 μg/ml of anticapsular antibody reached an absorbence of 0.3. For IgM, a 1:300 dilution of a serum containing 6.6 μg/ml was used. In previous studies, replicate values obtained in assays performed on different days agreed within a onefold dilution in 90% of samples (7).

Total IgG and IgM concentrations were measured in serum samples obtained prior to immunization from the 56 children in St. Louis using the technique of radial immunodiffusion (ICL Scientific, Fountain Valley, CA).

The Km(1) and Gm(23) immunoglobulin allotypes were measured using techniques of hemagglutination inhibition using reagents and methods that have been described in detail (35, 36). The assays were performed on serum samples that had been stored frozen at −70°. The serum samples were coded and the clinical status of the subjects was not known to the technician or investigator.

Statistical analysis. Statistical analysis was performed using SPSS software (37) on a Harris 500 computer (Harris Corp., Melbourne, FL). The frequencies of the Km(1) or Gm(23) allotypes among the patients and controls were compared using the chi-square test. Odds ratios and 95% confidence intervals were calculated according to the method described by Haldane (38). A *t* test for unpaired samples was used to compare the geometric mean concentrations of antibody in vaccinees grouped according to Km(1) or Gm(23) immunoglobulin allotype status. Age is correlated with immune response to the type b polysaccharide (24). Because subjects with different allotypes were not individually matched for age, covariance analysis of the group data was used. Accordingly, differences in group geometric means [e.g.,

between Km(1) positive vs. Km(1) negative children] were tested by statistically removing the linear effect of age (the covariate). This is equivalent to testing for a difference in means of residuals. The residuals are the difference of the actual immune response values and a regression quantity based on the associated age variable (39).

Results

Km(1) allotype and response to immunization. In a previous report the antibody responses of infants from Jamaica immunized with type b polysaccharide-pertussis vaccine were examined (29). In a pilot study for the present work, we measured Km(1) allotypes in serum samples remaining from nine black Jamaican infants who had been immunized at 2, 4, and 6 mo of age. (Serum samples from the children in Jamaica were kindly provided by Christine Williams, MD, Lederle Laboratories, Pearl River, NY.) As shown in Table I, we found that children with Km(1) allotype had sixfold higher anticapsular antibody concentrations one month after the third injection of vaccine compared with the responses of those who lacked this allotype. This difference was not statistically significant (P = 0.09) but the sample size was small and the statistical power to detect a difference was limited. The data were sufficient to stimulate us to examine this same question in a larger group of children immunized with this vaccine in the United States. Therefore, we determined Km(1) and Gm(23) allotypes in children 9–30 mo of age living in St. Louis, MO, or Danbury, CT, who had received one injection of vaccine (Table I). We found that there were no significant differences in the serum concentrations of antibody before immunization between children who were Km(1) positive and those who were Km(1) negative. However, 1 mo after immunization, the anticapsular antibody concentrations were 4.6- to 9-fold higher in those with the Km(1) allotype ($P < 0.04$ for black children and $P < 0.03$ for white children, analyzed separately; t test). Because of the range in ages of these children, we performed an analysis of covariance in which we removed the effect of age at the time of immunization. The probability values for the main effect due to Km(1) on response to vaccine were 0.04 for black children, 0.06 for white children, and 0.004 for black and white children analyzed together.

In an effort to characterize further the antibody responses of Km(1) positive and Km(1) negative children to type b polysaccharide-pertussis vaccine, we measured class specific antibody against type b capsular polysaccharide in pre- and postimmunization sera from 25 of the subjects from St. Louis who were 9–23 mo of age, using an enzyme-linked immunosorbent assay. We found no significant differences in the IgM anticapsular antibody titers 1 mo after immunization in the seven children with Km(1) compared with those in 18 children who lacked this allotype ($F = 0.5$, $P = 0.5$ by analysis of covariance). However, children with Km(1) had significantly higher IgG anticapsular antibody responses than those who lacked Km(1) (F = 10.7, P = 0.004 by similar analysis). The higher anti-capsular antibody responses of children with Km(1) thus appeared to be primarily IgG and not IgM.

To determine whether the higher antibody responses of children with the Km(1) allotype reflected higher total concentrations of serum Ig, we also measured the IgG and IgM concentrations in serum obtained before immunization from

Table I. Anticapsular Antibody Responses to PRP-Pertussis Vaccine in Relation to the Km(1) Ig Light Chain Allotype

				Serum anticapsular antibody (*ng/ml*)				
				Preimmunization		Post (1 mo)		
Population	Km(1)	Number tested	Mean age at time of injection	Geo mean	Mean $\log_{10}$±SD	Geo mean	Mean $\log_{10}$±SD	P*
			mo					
Blacks, Jamaica, West Indies‡	+	4	6	110	2.04±0.65	2,161	3.34±0.14	0.09
	−	5	6	168	2.36±0.32	358	2.55±0.41	
Blacks, St. Louis, MO§	+	13	22	109	2.04±0.51	1,702	3.23±0.66	<0.04
	−	8	21	64	1.80±0.45	366	2.56±0.61	
Whites, St. Louis, MO and Danbury, CT§	+	5	20	71	1.85±0.53	5,176	3.72±0.53	<0.03
	−	48	17	95	1.98±0.82	543	2.73±0.96	

* There were no significant differences in the antibody concentrations prior to immunization with respect to Km(1) allotype. The probabilities of the differences in the geometric mean antibody concentrations observed after immunization were calculated by a t test (two-tailed). Analysis of covariance was also performed on the data from blacks in St. Louis and from all white subjects to remove the effect of age. The probability values for the main effect due to Km(1) on antibody response were P = 0.04 for blacks, P = 0.06 for whites, and $P < 0.004$ for blacks and whites combined. ‡ Immunization at 2, 4, and 6 mo of age. Neither Km(1)+ or Km(1)− infants responded to the first two injections. For our analysis, antibody was measured in serum obtained prior to the last injection and 1 mo later. § Immunization once at 9–30 mo of age. Serum obtained for antibody before immunization and 1 mo later.

the 56 vaccinees in St. Louis. As shown in Table II, no significant differences were found when the mean serum Ig concentrations were compared between children with Km(1) and those who lacked this allotype.

Gm(23) allotype and response to immunization. The antibody responses of the white children from St. Louis and Danbury also were analyzed in relation to Gm(23). (Black children were excluded because, as expected, only one of the black vaccinees was positive for this marker.) There were 27 white children who were Gm(23) positive and 26 who were Gm(23) negative. The mean ages of the two groups were 18.5 and 16.6 mo, respectively ($P > 0.4$). There were no significant differences between the anticapsular antibody responses to immunization of the two groups (geometric mean serum anti-PRP antibody concentrations 1 mo after immunization of 1,005 and 444, respectively, $P = 0.2$ by t test, or $P = 0.4$ by analysis of covariance with age as a covariate).

Frequency of Km(1) or Gm(23) in patients with Haemophilus disease. As summarized in Table III, Km(1) was detected in only 38% of black patients with *Haemophilus* meningitis but in 81% of those with epiglottitis ($\chi^2 = 9.94$, $P < 0.002$) and 66% of black control subjects ($\chi^2 = 11.4$, $P < 0.0007$, when compared with that in patients with meningitis). Black children with Km(1) had a 3.2-fold lower relative risk of developing meningitis than those who lacked this allotype (odds ratio = 0.32, 95% confidence interval 0.3 to 0.6). However, blacks with Km(1) did not have a lower risk of epiglottitis (odds ratio = 2.0, 95% confidence interval 0.6 to 6.2). In contrast to blacks, the risk of meningitis was not decreased in white subjects with the Km(1) allotype (Table III).

There were no significant differences in the frequency of Gm(23) among patients with meningitis, epiglottitis, or controls (for blacks: 11, 11, and 13%, respectively; for whites: 73, 70, and 66%, respectively; $P > 0.5$).

Discussion

The purified type b polysaccharide is a poor immunogen in children < 18–23 mo of age (24). In this study, we used a vaccine consisting of the type b polysaccharide mixed with pertussis vaccine (27). This vaccine is immunogenic in rats (27, 28), a species that does not respond to the purified polysaccharide. This vaccine also is immunogenic in most human infants 12–23 mo of age (7, 29, 30), an age group highly susceptible to type b *Haemophilus* disease (1–3). In the present study, 77% of children given the combined vaccine produced antibody, including in many, IgG. This high rate of response permitted analysis of the subjects' responses in relation to the presence or absence of the Km(1) or Gm(23) allotypes.

We found that both black and white children who had the Ig light chain allotype, Km(1), developed higher antibody responses to the type b polysaccharide after immunization with this preparation than did children who lacked Km(1). The higher responses reflected different IgG anticapsular antibody responses; the IgM responses of Km(1) positive and negative children were not significantly different.

Although our data do not address the mechanism of the higher responses of the children with Km(1), two other studies provide information suggesting that the higher responses may not be capsular antigen specific, but instead be modulated by different genetically regulated responses to the adjuvant (15, 17). In a study of the responses to a polysaccharide vaccine administered with an adjuvant (group B meningococcal polysaccharide "complexed" with an outer membrane protein), adults with the Km(1) allotype had higher antipolysaccharide antibody responses than those who lacked Km(1) (17). As in the present study, this finding was present in both blacks and whites. In contrast, in a preliminary study of children immunized with the purified type b *Haemophilus* polysaccharide, lower anticapsular antibody responses were present among white children with Km(1) compared with those who lacked Km(1) (16). (In this latter study, no differences in response

Table II. Serum Immunoglobulin Concentrations in Relation to the Km(1) Allotype

Km(1)	Number tested*	Mean age	Ig concentrations±SD		
			IgM	IgG	*P*
		mo	*mg/dl*	*mg/dl*	
+	17	21.4	207±92	1082±317	NS‡
−	39	18.3	202±80	920±353	NS

* Includes all vaccinees from St. Louis ($n = 56$). Data from blacks and whites are combined.

‡ NS, not significant ($P > 0.05$ by t test and by analysis of covariance with age as covariate).

Table III. Frequency of Km(1) Immunoglobulin Allotype in Patients with Haemophilus Influenzae Type b Meningitis or Epiglottitis

	Number tested	Number with Km(1)	Odds-ratio	χ^2	*P**
		%			
Blacks					
Meningitis	66	25 (38)	0.3	11.4	<0.0007
Epiglottitis	21	17 (81)	2.0	1.4	NS‡
Controls	83	55 (66)	—	—	—
Whites					
Meningitis	104	19 (18)	1.0	0.1	NS
Epiglottitis	50	10 (20)	1.2	0.1	NS
Controls	90	16 (18)	—	—	—

* Compared with frequency of Km(1) in corresponding control group. $\chi^2 = 9.94$ (Haldane correction for small sample size), $P < 0.002$, comparing frequency of Km(1) in patients with meningitis with that in patients with epiglottitis.

‡ NS, not significant ($P > 0.05$).

were observed with respect to Km[1] among black children, but there were too few black children of either allotype who responded to vaccine to permit an appropriate analysis.)

Antibody responses to many bacterial polysaccharide antigens are thought to be thymic independent (40, 41). In humans, both IgM and IgG responses to polysaccharide antigens are observed (23, 42) but it appears that the IgG responses may preferentially involve the IgG_2 subclass (25). Siber et al. (43) have provided data that the serum pool size of IgG_2 in an individual may be an important determinant of the magnitude of the antibody response to certain bacterial polysaccharides, including the type b capsule of *Haemophilus* (43). Therefore, we measured IgG_2 concentrations in serum samples obtained before immunization from the subjects in this trial (44), using a sensitive and specific inhibition radioimmunoassay (45). Anticapsular antibody responses to vaccine were found to correlate with serum IgG_2 concentrations ($r = 0.3$, $P < 0.02$). However, after correction for age, this variable (IgG_2) no longer contributed significantly ($P > 0.10$). Thus, although IgG_2 pool size may contribute, its effect appears to be confounded with age.

In the United States, black children have a three- to fivefold higher risk of developing *Haemophilus* meningitis than white children (1–3). The present data suggest that the risk of developing *Haemophilus* meningitis among blacks is not uniform but is higher among the 40–50% of blacks who lack the Km(1) allotype (Table III). It should be noted that this finding was not present in whites (Table III). In our study, race was defined by social criteria; that is, subjects were categorized according to the race indicated by their parents. However, from previous studies, many North American blacks have evidence of black-white racial admixture (46). One method to estimate the proportion of genes that are of caucasian origin is to use the Gm locus, since certain alleles such as Gm(2), Gm(3), or Gm(21) are prevalent in whites but are absent in African blacks (47). It was therefore of interest to compare the frequency of Km(1) among the black patients and controls after further subdividing them into those with African black Gm phenotypes, i.e., (1,17;5,13), (1,17;5,6,13), or (1,17;5,6) or those with evidence of black-white admixture [presence of Gm(2), Gm(3), or Gm(21)]. In both groups, the respective frequency of Km(1) was significantly lower in patients with meningitis than in controls (for black subjects with black African phenotypes, 41 vs. 65%, respectively ($\chi^2 = 6.4$, $P < 0.01$); for black subjects with Gm(2), Gm(3), or Gm(21), 35 vs. 62%, respectively ($\chi^2 = 3.84$, $P = 0.05$). Thus, although the frequency of Km(1) was similar among white patients with meningitis and controls, it was significantly lower in the black patients with meningitis compared with that in black controls, irrespective of whether the Gm phenotype of the blacks contained alleles derived from whites.

It has been demonstrated previously that white patients with epiglottitis have different frequencies of certain erythrocyte MNSs antigens than white patients with meningitis (5, 6). Similarly, in the present study, we have shown that black patients with meningitis or epiglottitis differ from each other genetically with respect to the frequency of the Km(1) allotype (Table III). The fact that this finding was not present in whites in our study suggests that the immunogenetic background of white patients with epiglottitis or meningitis may be different from that of black patients.

We found no significant differences in the frequency of the Gm(23) allotype among the patient groups and controls, failing to confirm a preliminary report of a lower frequency of this allotype in patients with *Haemophilus* disease (19). The reason for the discrepant results in the two studies is not evident. Serum samples have been exchanged and there was complete concordance in the results of typing for Gm(23) between the two laboratories (authors' unpublished observation).

In summary, our data relating magnitude of the antibody response to *Haemophilus* type b polysaccharide-pertussis vaccine and the Km(1) allotype provide further evidence supporting genetic regulation of certain immune responses in humans. Furthermore, the lower risk of *Haemophilus* meningitis found in black children with the Km(1) allotype also suggests that with an appropriate environmental or genetic background, genes affecting immune responses may function to confer resistance or susceptibility to an infectious agent. One implication of our data is that a vaccine may be immunogenic in the majority of children of susceptible age, but have only limited efficacy in preventing disease in the general population because of failure to confer protection to the subpopulation of children at highest risk of disease because of genetic factors.

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