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Research Article

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Correlation between G2m(n) Immunoglobulin Allotype and Human Antibody Response and Susceptibility to Polysaccharide Encapsulated Bacteria

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Abstract

To determine whether genetic factors influence the human antibody response to polysaccharides, we correlated Ig allotypes with the concentrations of antibody to 14 bacterial capsular antigens in 130 actively immunized Caucasian adults. The 88 individuals possessing G2m(n), an allotype antigen of IgG_2 subclass heavy chains, had significantly higher postimmunization antibody levels to *Haemophilus influenzae* type b (Hib) and 8 of 11 pneumococcal types ($P < 0.05$) than the 42 lacking this antigen. For Hib, pneumococcus type 14, and meningococcus group C, an increased response was observed in IgG class but not in IgM or IgA classes of antibody. The G2m(n) positive individuals also had higher preimmunization antibody levels to most polysaccharide antigens. Total IgG_2 concentrations were correlated with the mean postimmunization antibody concentrations to pneumococci ($P = 0.005$), but this correlation was independent of G2m(n) by multiple regression analysis.

To determine if the lack of G2m(n) was associated with increased susceptibility to infection, we compared the frequencies of various Ig allotypes in 98 children infected with Hib and 98 matched controls. Caucasian children with Hib infections other than epiglottitis were significantly more likely to lack the G2m(n) allotype than controls ($P < 0.05$). G2m(n) negative Caucasian children ≤ 18 mo old have a 5.1-fold higher risk of nonepiglottitic Hib infections than G2m(n) positive children ($P < 0.01$). We conclude that allotypic variants of the gamma-2 heavy chain genes, or genes in linkage equilibrium with them, exert a regulatory influence on the caucasian antibody response to a variety of immunologically distinct bacterial polysaccharide antigens. Young Caucasian children of the low responder phenotype, i.e., those lacking the G2m(n) allotype, are genetically predisposed to Hib and perhaps other bacterial infections.

Introduction

Haemophilus influenzae type b (Hib),[1] pneumococci, and meningococci are major bacterial pathogens. Antibodies to the surface structures of these bacteria, particularly to the capsular polysaccharides, are important in protection against infection (1–4). Several lines of evidence suggest that the antibody response to polysaccharide antigens and susceptibility to these bacterial infections may be under genetic control. Children who have recovered from meningitis have lower anti-Hib antibodies than age matched siblings (5) and do not respond to immunization as well as controls (6). Siblings of patients with meningitis have lower antibody responses to a Hib-pertussis complex vaccine than control children (7). Certain ethnic populations such as native Americans (8) and Alaskan Eskimos (9) have an extremely high incidence of infection by Hib and pneumococci. Hib disease expression also differs in various races. For example, epiglottitis is common in Caucasian and rare in Black, Eskimo, and native American children. There are differences between children with epiglottitis and healthy children in MNS erythrocyte antigen phenotypes (5). Although socioeconomic and epidemiologic factors may explain some of these observations, genetic factors are also likely to be important.

We have previously shown that the human antibody response to bacterial polysaccharide antigens is correlated with the individual's total IgG_2 subclass concentration (10). The majority of human IgG antibody to certain polysaccharide antigens is of this subclass (11, 12). The concentrations of IgG_2 as well as IgG_3 and IgG_4 are higher in individuals with particular Ig heavy chain antigens called allotypes (13–15). Allotypes are genetically determined variants of Igs that provide a tool for examining the influence of genes located in or near the heavy or light chain loci on the antibody response. In mice (16, 17) and in humans (18–20), Ig allotype antigens have been associated with increased or decreased antibody responses to a variety of antigens.

In this study we examined the relationship between human Ig allotype antigens and the antibody concentrations of healthy Caucasian adults before and after immunization with 14 polysaccharide antigens. We were particularly interested in the G2m(n) allotype because of its location on the heavy chain of IgG_2 subclass Igs. In the second phase of the study we compared the Ig allotypes of children with Hib infections and matched controls to determine whether Ig allotypes were correlated with the risk of infection.

Methods

Study population (active immunization). 58 male and 72 female Caucasian plasma donors with a mean age of 33 yr were immunized with 0.5 cc 14-valent pneumococcal vaccine (Pneumovax, Lot 1912B, Merck, Sharp and Dohme, West Point, PA) and concurrently with 0.5 cc Hib vaccine (Lot 764-CF320; Merck, Sharp and Dohme) and 0.5

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1. *Abbreviations used in this paper:* CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; Hib, *Haemophilus influenza* type b.

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cc bivalent meningococcal vaccine (Menomune A/C, Connaught Laboratories, Swiftwater, PA), combined in the same syringe. Serum for Ig allotyping, Ig concentrations, and polysaccharide antibodies was obtained before and 4 wk after immunization and stored at −20°C until assay.

Study population (disease susceptibility). 98 children with Hib disease seen at Children's Hospital Medical Center, Boston, MA from 1977 to 1982 were studied. Their clinical features are summarized in Table I. The case definition of Hib infection was the isolation of Hib from the blood, cerebrospinal fluid (CSF), or other sterile body fluid ($n = 79$), or the detection of Hib capsular antigen in blood and/or CSF in the presence of a clinical syndrome of meningitis, epiglottitis, cellulitis, or septic arthritis ($n = 19$). Patients with known immunodeficiency syndromes and sickle cell disease were excluded. Because passive maternal antibody may interfere with allotyping, infants aged <6 mo were also excluded.

Controls were chosen from children with other febrile illnesses observed during the same period, matched to cases by age, sex, race, and neighborhood of residence using the postal code. Individuals with prior culture documented Hib, pneumococcal, or meningococcal infection were excluded. The controls had clinical diagnoses of upper respiratory infections ($n = 10$), lower respiratory infections ($n = 26$), viral syndromes ($n = 18$), fever without a focus ($n = 11$), aseptic meningitis ($n = 4$), nonfacial cellulitis ($n = 4$), and other febrile conditions ($n = 25$).

Assays. Antibody to the Hib capsular polysaccharide was measured by radioimmunoassay (21) with tritiated capsular polysaccharide kindly provided by Dr. Porter Anderson (University of Rochester, Rochester, NY). The assay was standardized with a standard serum (S. Klein) supplied by Dr. John Robbins (National Institutes of Health, Bethesda, MD).

Antibody to the other polysaccharides was quantitated by radioimmunoassay using radiolabeled antigens from 11 pneumococcal vaccine types (22) in Dr. Schiffman's laboratory, and meningococcal serogroups A and C (23) in Dr. Gotschlich's laboratory.

Class specific antibody to the capsular polysaccharides of Hib, meningococcus group C, and pneumococcus type 14, was quantitated by enzyme-linked immunosorbent assay (ELISA) using the respective antigens coupled to tyramine with cyanogen bromide by the procedure of R. Insel (University of Rochester, Rochester, NY). Briefly, 50 mg cyanogen bromide was added to 1 mg of polysaccharide in 1 ml 0.1 M $NaHCO_3$, pH 10.2, and the pH was maintained between 10.0 and 10.2 with 0.1 N NaOH. After 6 min, 0.4 mg tyramine in 1 ml 0.1 M $NaHCO_3$, 0.5 M NaCl, pH 8.5, was added. The mixture was incubated at room temperature for 1 h and at 4°C for 24 h and dialyzed against phosphate-buffered saline. Wells of polystyrene microtiter plates (Flow Laboratories, Inc., McLean, VA) were coated with 100 μl of tyraminated capsular polysaccharide at 1 μg/ml for 90 min at 37°C and were washed with wash buffer (phosphate-buffered saline, 0.05% Tween). 100 μl of serum diluted in wash buffer was added per well, incubated at room temperature for 24 h, and washed. 100 μl of appropriately diluted alkaline phosphatase conjugated IgG fractions of goat anti-human IgG, IgM, or IgA (Atlantic Antibodies, Scarborough, ME) were added, incubated at 37°C for 3 h, and washed. Wells were developed with 100 μl of 1 mg/ml paranitrophenyl phosphate disodium (Sigma Chemical Co., St. Louis, MO) in 1 M diethanolamine, 0.5 mM $MgCl_2$, 0.01% NaN_3, pH 9.8 for 1 h at room temperature. The reaction was stopped by adding 75 μl 1 N NaOH and the OD at 405 nm was read in an ELISA reader (model EL 307 IP; Biotech Inc., Burlington, VT). The assay was standardized by the method of Zollinger and Boslego (24) using a human hyperimmune plasma pool (25) as a reference standard.

Table I. Clinical Characteristics of Patients with Hib Infection

	No. of patients in each age group		
	6–18 mo	>18 mo	All ages
Total no.	36	62	98
Sex			
Male	21	28	49
Female	15	34	49
Race			
Caucasian	27	46	73
Black	6	12	18
Hispanic	3	4	7
Focus of infection			
Meningitis	27	20	47
Epiglottitis	3	27	30
Cellulitis	5	9	14
Bacteremia	1	3	4
Arthritis	0	3	3

The concentrations of serum IgG, IgM, and IgA were assayed by nephelometry (26) and IgG_2 subclass was assayed by radial immunodiffusion (27).

Ig allotypes were determined by hemagglutination inhibition in the laboratory of Dr. E. Van Loghem. The following allotypic markers were identified: G1m(z), G1m(a), G1m(x), G1m(f), G2m(n), G3m(g) including g1g5, G3m(b) including b0b1b3b4b5, G3m(s), G3m(t), G3m(c3), G3m(c5), A2m(1), A2m(2), Km(1), and Km(3). Anti-Rh antibodies were used as the coating antigens except for G2m(n), A2m(1), and A2m(2), where erythrocytes were coated with isolated myeloma proteins using $CrCl_3$ (28).

Statistical methods. Data organization and analysis were performed on the PROPHET system, a national computer system sponsored by the Chemical/Biological Information Handling Program, National Institutes of Health. Before statistical analysis, antibody concentrations were converted to logarithms, which usually normalized their distributions (29). The geometric mean pneumococcal antibody response, which gives an estimate of the individual's overall pneumococcal antibody response, was defined as the geometric mean of that individual's antibody concentrations to the 11 individual types measured (29). Because this measure is usually normally distributed, it was not converted to logarithms for further statistical analysis.

Means were compared by the two-tailed t test for normally distributed values and by the Mann-Whitney test for non-normal values. Correlations, covariance analyses, and multiple linear regression analyses were performed with the logarithms of the antibody concentrations. Allotypes and allotype interactions were analyzed by assigning dummy variables for presence ($n = 1$) or absence ($n = 0$) of each allotype or interaction. In the disease susceptibility study the population was stratified by race, focus of infection, and age; and differences in the distribution of allotypes were analyzed by multiple linear logistic regression.

Results

Correlations of human antibody responses to specific polysaccharides. A correlation matrix summarizing the correlation coefficients (r values) between the postimmunization antibody responses to the 11 immunochemically distinct pneumococcal polysaccharides is summarized in Table II. The correlations are uniformly positive and significant for the majority of polysaccharides. This indicates that individuals who are low-responders to one antigen, tended to be low-responders to most of the others, and conversely, that high-responders to one antigen tended to be high responders to all the others.

Immunoglobulin allotypes and response to immunization. The distributions of the Gm, Am, and Km phenotypes of the

Table II. Correlation Coefficients between Postimmunization Antibody Concentrations to 11 Pneumococcal Serotypes in 130 Healthy Adults*

	Pneumococcus type										
	1	3	6	7	8	9	12	14	18	19	23
Pneumococcus type											
1	1.00	—	—	—	—	—	—	—	—	—	—
3	0.30	1.00	—	—	—	—	—	—	—	—	—
6	0.43	0.33	1.00	—	—	—	—	—	—	—	—
7	0.49	0.51	0.56	1.00	—	—	—	—	—	—	—
8	0.37	0.49	0.46	0.63	1.00	—	—	—	—	—	—
9	0.25	0.53	0.27	0.58	0.62	1.00	—	—	—	—	—
12	0.49	0.26	0.52	0.49	0.51	0.43	1.00	—	—	—	—
14	0.11	0.08	0.29	0.34	0.32	0.31	0.51	1.00	—	—	—
18	0.37	0.32	0.33	0.48	0.45	0.42	0.57	0.32	1.00	—	—
19	0.22	0.24	0.33	0.37	0.42	0.42	0.51	0.41	0.32	1.00	—
23	0.21	0.15	0.31	0.31	0.30	0.40	0.44	0.45	0.44	0.40	1.00

* Correlation coefficients > 0.17 are significant at $P < 0.05$; correlation coefficients > 0.22 are significant at $P < 0.01$.

130 immunized adults are shown in Table III. This distribution is very similar to that reported by Morell et al. (13) in Swiss volunteers and by Fraser et al. (30) in the Dutch population. The Gm allotypes are borne on closely linked IgG_1, IgG_2, and IgG_3 heavy chain constant region genes on chromosome 14 and are thus inherited as groups termed haplotypes (28). The most common Caucasian Gm haplotypes are Gm(f,b), Gm(f,n,b), Gm(z,a,g), and Gm(z,a,x,g). The phenotypes Gm(f,n,b;z,a,g), Gm(f,b;z,a,g), Gm(f,b), Gm(f,n,b;z,a,x,g), Gm(f,b;z,a,x,g), and Gm(z,a,g) permit inference of the genotype (Table III). Individuals with the Gm(f,n,b) or Gm(z,a,x,g) phenotype, on the other hand, are ambiguous because they could be homozygous or heterozygous for the G2m(n) or G1m(x) allotypes, respectively. For example, the genotypes Gm(f,n,b;f,b) and Gm(f,n,b;f,n,b) would both have the phenotype Gm(f,n,b).

Table III. Gm, Am, and Km Phenotypes of 130 Immunized Adults

Phenotypes*	No.	Percent
Gm phenotypes		
Gm(f,n,b)	49	38
Gm(f,n,b;z,a,g)	27	21
Gm(f,b;z,a,g)	13	10
Gm(z,a,x,g)	10	8
Gm(f,b)	9	7
Gm(f,n,b;z,a,x,g)	9	7
Gm(z,a,g)	7	5
Gm(f,b;z,a,x,g)	2	2
Others	4	3
Am phenotypes		
1 + 2−	114	88
1 + 2+	13	10
1 − 2+	1	1
IgA_2 deficient	2	2
Km phenotypes		
1 + 3−	2	2
1 + 3+	12	9
1 − 3+	116	89

* The Gm allotypes are grouped in haplotype groups commonly found in Caucasians (see text).

The correlations of postimmunization polysaccharide antibodies with the Ig allotypes were analyzed by stepwise multiple linear regression (Table IV). The Gm allotypes were analyzed according to the common Caucasian haplotypes as noted above. Only the Gm(f,n,b) haplotype was significantly associated with postimmunization antibodies to Hib and pneumococci. Since G2m(n) is almost always associated with G1m(f) and G3m(b), we will subsequently refer to the Gm(f,n,b) haplotype as G2m(n) or n. After the effect of Gm(f,n,b) was removed from the regression model, Gm(f,b), Gm(z,a,g), Gm(z,a,x,g), A2m(2), and Km(1) had no significant independent association with the geometric mean antibody concentrations to Hib, meningococcus A or C, or the mean of 11 pneumococcal types before or after immunization.

G2m(n) allotype and response to immunization. The 88 G2m(n) positive individuals had equal or higher mean antibody concentrations to all 14 polysaccharide antigens both before and after immunization than the 42 G2m(n) negative individuals (Table V). After immunization, mean antibody concentrations to Hib and to 8 of 11 pneumococcal antigens differed significantly. Even if Bonferroni's adjustment for multiple independent comparisons (31) is applied, differences between three of the pneumococcal types (types 12, 19, and 23) remain significant at $P < 0.05$. Since the measurements are not independent, as shown by the strong correlations between antibody concentrations to different types (Table II), Bonferroni's adjustment is an excessively stringent criterion for significant differences. A better way to compare a large number of mutually correlated observations is to examine an aggregate estimate of the antibody response. When this is done by calculating each individual's geometric mean antibody concentration to the 11 pneumococcal types, all of which are assayed in the same laboratory by the same technique (22), a highly

Table IV. Correlation of Allotype Haplotypes and IgG_2 Levels with Anti-polysaccharide Antibody Concentrations after Immunization

Marker	Partial correlation coefficients* (*P* value) for antibodies to: *H. influenzae* type b	*N. meningitidis* group A	*N. meningitidis* group C	*Pneumococcal*‡ geom. mean
Gm(f,n,b)	+0.20 (0.03)	+0.08	+0.12	+0.31 (0.001)
Gm(f,b)	−0.07	−0.10	−0.08	−0.04
Gm(z,a,g)	−0.15	−0.05	+0.08	−0.03
Gm(z,a,x,g)	+0.11	−0.10	−0.02	−0.06
Am(2)	−0.09	−0.04	−0.04	+0.17
Km(1)	−0.15	−0.14	−0.11	0.06
Gm(f,n,b) × Km(1)§	+0.02	+0.11	+0.01	−0.02
IgG_2 concentration	+0.07	+0.12	+0.13	+0.26 (0.005)

* Partial correlation coefficients were obtained by multiple linear regression analysis. The coefficients given are calculated with the significant variables included in the regression model. ‡ Geometric mean of the 11 capsular polysaccharide types measured (see text). § Interaction of Gm(f,n,b) and Km(1). A dummy variable was assigned so that individuals having Gm(f,n,b) and lacking Km(1) were +1 and the remaining individuals were 0.

significant difference is found between G2m(n) positive and negative groups ($P = 0.0005$).

When preimmunization antibody levels are compared, antibody to pneumococcal type 8 ($P = 0.044$) and pneumococcal type 23 ($P = 0.011$) differed significantly, while five others showed trends ($P < 0.10$) (Table V).

To determine which classes of antibody were correlated with G2m(n), we measured the class specific antibody response to capsular polysaccharides of Hib, pneumococcus type 14, and meningococcus group C. After immunization, G2m(n) positive individuals had significantly higher mean IgG class antibody concentrations for all three antigens than G2m(n) negative individuals (Table VI). No differences were noted for postimmunization IgM or IgA concentrations.

Since preimmunization and postimmunization antibody concentrations were weakly but significantly correlated for all of the polysaccharides measured, we also examined correlations of the G2m(n) allotype by using other measures of antibody response, such as the fold-rise (post/pre) and increase (post minus pre) in antibody concentrations, as well as by using

Table V. Relationship between the G2m(n) Allotype and Anti-polysaccharide Antibody Concentrations before and after Immunization

	Geometric mean antibody concentrations (*P* value)*			
	Before immunization	Before immunization	After immunization	After immunization
Antibody to (*U*):	G2m(n)+ (*n* = 88)	G2m(n)− (*n* = 42)	G2m(n)+ (*n* = 88)	G2m(n)− (*n* = 42)
H. influenzae type b (*μg/ml*)	2.17	1.70	35.0	21.3 (0.024)
N. meningitidis (*μg/ml*)				
Group A	3.55	3.61	12.1	11.8
Group C	0.878	0.869	33.9	26.5
S. pneumoniae (*ng protein N/ml*)				
Type 1	1,010	874	2,100	1,660
3	152	116	1,930	1,260
6	211	144	723	445 (0.035)
7	438	177 (0.071)	2,040	955 (0.007)
8	555	434 (0.044)	1,530	1,030 (0.022)
9	72	55	476	288
12	1,760	985 (0.055)	3,440	2,150 (0.001)
14	501	293 (0.090)	1,690	1,010 (0.007)
18	700	361 (0.077)	2,540	1,460 (0.006)
19	167	100 (0.076)	1,050	334 (0.0001)
23	931	632 (0.011)	3,020	1,430 (0.001)
Geometric mean of types	520	423	1,870	1,220 (0.0005)

* A two-tailed *t* test was used for normally distributed values and a Mann-Whitney test was used for nonnormally distributed values.

Table VI. Relationship between the G2m(n) Allotype and Class-specific Postimmunization Antibody Concentrations to Hib and Pneumococcus Type 14 and Meningococcus Group C Capsular Polysaccharides Measured by ELISA

	Geometric mean antibody concentrations (*P* value)	
Antibody to (*U*):	G2m(n)+ (*n* = 88)	G2m(n)− (*n* = 42)
Hib (*ng/ml*)		
IgG	12,950	5,735 (0.005)
IgM	1,425	1,201
IgA	1,408	1,425
S. pneumoniae type 14 (*ng/ml*)		
IgG	36,300	21,200 (0.014)
IgM	3,870	5,150
IgA	532	630
N. meningitidis group C (*ng/ml*)		
IgG	5,140	2,430 (0.012)
IgM	1,690	1,890
IgA	557	630

covariance analysis with the preimmunization concentration as the covariate. The G2m(n) allotype effect remained significant by each of these methods (data not shown).

A gene dose effect was sought by comparing the antibody concentrations in G2m(n) negatives, known G2m(n) heterozygotes, and individuals with the Gm(f,n,b) phenotype. In Caucasian populations, individuals in the last group have a 50% chance of being true homozygotes, i.e., Gm(f,n,b;f,n,b) (15). The corresponding postimmunization mean pneumococcal antibody concentrations in these three groups were 1,217 ng/ml, 1,785 ng/ml, and 1,956 ng/ml, consistent with a gene-dose effect for G2m(n). A gene dose effect also predicts that individuals with the Gm(f,n,b) phenotype should have a bimodal distribution in their postimmunization antibody responses, the higher mode corresponding to the G2m(n) homozygotes, and the lower corresponding to the heterozygotes. A bimodal distribution was indeed found for the mean pneumococcal antibody concentrations after immunization (Fig. 1). The antibody concentration of the lower mode approximates that of the G2m(n) heterozygotes above.

Frequency of G2m(n) negative phenotype in high and low responders to pneumococcal polysaccharides. The frequency of the low-responder or G2m(n) negative phenotype was compared

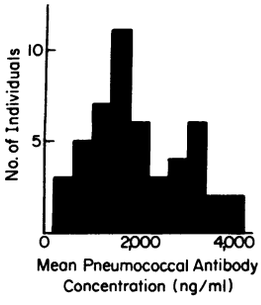

Figure 1. Distribution of the mean postimmunization antibody concentrations to 11 pneumococcal polysaccharides in 49 individuals with the Gm(f, n, b) phenotype.

Table VII. Frequency of the G2m(n) Negative Phenotype in Groups with High or Low Responses to 11 Pneumococcal Polysaccharides

Percentile of geometric mean pneumococcal antibody concentration	G2m(n) negatives/total	Percent
After immunization		
Whole population	42/130	33
Highest 10%	1/13	8
Highest 25%	5/32	16
Second 25%	7/33	21
Third 25%	13/33	39
Lowest 25%	18/32	56
Lowest 10%	11/13	85

in subgroups of individuals who achieved the highest and lowest antibody concentrations to the 11 pneumococcal polysaccharides measured (Table VII). The low-responder phenotype was infrequent in the high responders and more frequent in low-responders when compared with the total population. The frequency differences were most striking in the top and bottom 10% groups.

Ig concentrations. Total IgG, IgM, and IgA concentrations did not correlate significantly with anti-polysaccharide antibody levels. However, the IgG_2 subclass concentration was significantly correlated with the postimmunization mean pneumococcal antibody concentration ($r = 0.26$) ($P < 0.005$). This correlation was not reduced when the effect of G2m(n) was removed from the multiple regression model as summarized in Table III. As expected from this observation, G2m(n) positive individuals did not have significantly higher IgG_2 levels than G2m(n) negatives (geometric means of 1.42 and 1.46 mg/ml, respectively).

IgG, IgM, and IgA concentrations were analyzed in relation to the Ig allotypes. Km(1) positive individuals had a lower mean IgM level than Km(1) negatives (0.65 mg/ml vs. 0.88 mg/ml, respectively, $P < 0.02$). Other allotype antigens were not correlated with IgG, IgM, or IgA levels.

Frequency of G2m(n) negative phenotype in children with invasive Hib disease and matched controls. The frequency of the common Caucasian Gm haplotypes and Km allotypes in cases and controls are summarized in Table VIII. Only the G2m(n) marker was associated with disease when the whole population was analyzed (relative risk = 1.5, Table IX). Stratification by race revealed that the G2m(n) marker was relatively uncommon (32%) and was equally distributed in non-Caucasian cases and controls. Among Caucasians analyzed as a whole, infected children were more likely to lack the G2m(n) marker than controls, but this difference did not reach statistical significance ($X^2 = 2.54$, $P = 0.11$) (Table IX). Stratified analysis by type of infection revealed that Caucasian children with epiglottitis had identical frequencies of the G2m(n) marker and therefore did not contribute to the difference between infected and control children. Caucasians with nonepiglottitic Hib infections were significantly more likely to lack the G2m(n) allotype than controls ($P < 0.05$). This difference was most striking in the group aged <18 mo ($P < 0.01$). The relative risk of Hib infection in low-responder [G2m(n) negative] versus high-responder [G2m(n) positive] individuals was calculated to be 5.1-fold in this group.

*Table VIII. Frequency of Ig Gm and Km Phenotypes in Children with Hib Infections and Matched Controls**

	Infected		Control	
	6–18 mo	>18 mo	6–18 mo	>18 mo
Whole population	27 (9)	46 (16)	27 (9)	46 (16)
Gm phenotypes				
Gm(f,n,b)	9 (3)	18 (3)	15 (1)	14 (5)
Gm(f,n,b;z,a,g)	2 (1)	11 (1)	5 (1)	8 (0)
Gm(f,b;z,a,g)	3 (0)	4 (0)	0 (0)	2 (1)
Gm(z,a,x,g)	6 (0)	4 (3)	1 (0)	5 (3)
Gm(f,b)	3 (0)	4 (2)	1 (0)	2 (0)
Gm(f,n,b;z,a,x,g)	1 (0)	4 (0)	4 (1)	8 (0)
Gm(z,a,g)	0 (0)	0 (2)	0 (2)	5 (1)
Gm(f,b;z,a,x,g)	3 (0)	1 (0)	1 (0)	2 (0)
Others	0 (5)	0 (5)	0 (4)	0 (6)
Km phenotypes				
1, 1	0 (1)	0 (3)	0 (2)	0 (1)
1, 3	6 (5)	5 (5)	3 (1)	6 (5)
3, 3	21 (3)	41 (8)	24 (6)	40 (10)

* The number of Caucasians with each given phenotype is indicated outside of brackets, and the number of non-Caucasians, within brackets.

Discussion

The finding of highly significant correlations in an individual's antibody responses to a large number of immunologically non-cross-reactive polysaccharide antigens (Table II), provides strong support for the hypothesis that the human antibody response to this class of antigens is under common regulatory control. We have previously shown (10), and here confirm, that an individual's total IgG_2 level correlates directly with the postimmunization antibody concentrations to pneumococcal polysaccharides. The major finding of these studies is the correlation between the G2m(n) allotype, an antigenic marker on the heavy chain of IgG_2 subclass Ig and antibody concentrations to bacterial polysaccharide antigens. Individuals expressing the G2m(n) allotype had higher levels of antibodies to most polysaccharides than individuals lacking G2m(n). Although this association was most striking after immunization, significant correlations were also observed with natural preimmunization antibody levels to several pneumococcal types. For the three antigens examined for class-specific responses, Hib, pneumococcus type 14, and meningococcus group C, the increased antibody response was confined to the IgG class.

In the second phase of these studies we documented a significant correlation between the low-responding G2m(n) negative phenotype and risk of Hib infection in young Caucasian children. The low-responder phenotype did not correlate with Hib disease in non-Caucasians nor with epiglottitis in Caucasians. The latter finding is not surprising, since children with epiglottitis have normal or supranormal levels of antibody to Hib capsular polysaccharide (5). It is not clear why the increased susceptibility to Hib infection seems to be most marked in very young children. However, the capacity to make anti-polysaccharide antibody is marginal in children <18 mo of age, whereas adults, regardless of G2m(n) phenotype, uniformly had anti-Hib capsular polysaccharide levels above the protective threshold (>150 ng/ml) even before immunization. It therefore seems reasonable that the biologically significant effects of a genetically impaired antibody response would be manifest primarily in infancy.

Others have also noted correlations between Ig allotypes and human anti-polysaccharide antibody responses. Pandey et al. (18) have reported that Caucasian children with the Km(1) allotype had higher antibody responses to group C meningococcal vaccine and lower responses to Hib vaccine than Km(1) negative children. Recently, Granoff et al. (32) reported that both Caucasian and black children with the Km(1) marker had higher responses to immunization with a Hib-pertussis complex vaccine than children lacking Km(1), and that blacks lacking the marker were at increased risk for disease. Granoff et al. (32) also examined the G2m(n) marker (termed Gm[23] in their studies) and noted no significant differences in antibody responses. Our studies showed somewhat lower postimmunization antibody concentrations to Hib ($P = 0.13$), several pneumococcal types, and meningococcus C in individuals with the Km(1) allotype. The differences between these studies may relate to differences in the type of vaccine used to stimulate antibody responses, differences in the numbers of patients studied, or differences in age, race, or genetic make-up of the study populations.

Our studies do not address the mechanism whereby individuals who lack G2m(n) allotype produce decreased concentrations of IgG class antibodies to polysaccharides. Most of the bacterial polysaccharide antigens used in these studies are likely to be type 2 T-independent antigens. In the murine system, as in humans, these antigens elicit an IgG subclass restricted antibody response (33) that is influenced, at least in part, by genes mapping to the Ig heavy chain locus (16, 34). A subset of murine B cells bearing the Lyb-5 surface antigen responds to type 2 thymus independent antigens (35). A second surface antigen, Lyb-7, which maps to the Ig heavy chain locus (36), is necessary for Lyb-5 bearing cells to be activated by these antigens. An analogous system may be operative in humans.

A variety of other specific mechanisms could be responsible, including enhanced production of IgG_2 class antibody (37), or enhanced switching from IgM to IgG class antibody at the B cell level and allotype or idiotype mediated regulation of T cell interaction with B cells (38, 39). Genes linked to Ig heavy

Table IX. Frequency of the G2m(n) Negative Phenotype in Children with Hib Infections and Matched Controls

	G2m(n) negatives/total (percent)		Relative risk ratio*
	Infected	Controls	
			95% confidence interval
All patients	45/98 (46%)	36/98 (37%)	1.5
Non-Caucasians	17/25 (68%)	17/25 (68%)	1.0
Caucasians	28/73 (38%)	19/73 (26%)	1.8
Epiglottitis	8/25 (32%)	8/25 (32%)	1.0
Nonepiglottitis	20/48 (42%)	11/48 (23%)	2.4 (1.0–5.8)‡
6–18 mo	14/25 (56%)	5/25 (20%)	5.1 (1.5–17.3)§
19–36 mo	6/17 (35%)	4/17 (24%)	1.8
>36 mo	0/6 (0%)	2/6 (33%)	

* Relative risk ratio $= \frac{\text{G2m(n)} - \text{cases}}{\text{G2m(n)} + \text{cases}} \div \frac{\text{G2m(n)} - \text{controls}}{\text{G2m(n)} + \text{controls}}$.

‡ $X^2 = 3.86$, $P < 0.05$.

§ $X^2 = 6.88$, $P < 0.01$.

chain allotypes may also affect the rate of degradation of antigens by human macrophages. For example, individuals possessing both HLA-DR3 and the Gm(f,n,b) haplotype are slow degraders of sheep erythrocyte antigens (40). In murine systems, such slow antigen degradation is associated with high antibody responses.

The biological significance of allotype-linked hyporesponsiveness to bacterial polysaccharide antigens remains to be precisely defined. We have shown an increased risk of non-epiglottitic Hib infections in very young Caucasians. It is not clear whether the increased risks of infection extend also to other encapsulated pathogens such as the pneumococcus or to adult populations, particularly the aged, in whom anti-polysaccharide antibody responses may again be marginal (41). Finally, an important practical corollary of these observations is that evaluations of the immunogenicity of new bacterial polysaccharide vaccines in infants should carefully examine the antibody responses of G2m(n) negative individuals since they may develop a disproportionately high number of subsequent infections.

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