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**Research Article**

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# Correlation of the Km(1) Immunoglobulin Allotype with Anti-polysaccharide Antibodies in Caucasian Adults

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## Abstract

**Km allotype antigens are serologic markers expressed on kappa light chains of human immunoglobulins. To determine whether the Km phenotype of an individual is related to his ability to make antibodies to polysaccharide antigens, we correlated the Km allotypes of 129 healthy caucasian adults with the concentrations of specific antibodies to three bacterial polysaccharide antigens after immunization.**

**The 14 individuals expressing the Km(1) allotype had lower concentrations of IgG, IgM, and IgA antibodies by enzyme-linked immunosorbent assay and total antibody by radioimmunoassay to *Haemophilus influenzae* type b and *Neisseria meningitidis* group C capsular polysaccharides when compared with the 115 Km(1) negative individuals. The Km-associated differences in *H. influenzae* type b and *N. meningitidis* group C antibody concentrations were confined to kappa light chain–containing antibody ($P = 0.029$ and 0.003, respectively). Similarly, the Km(1) positives had slightly lower kappa chain–containing Ig than the Km(1) negatives ($P = 0.079$).**

**We conclude that genes in or near the kappa light chain locus play a role in the regulation of kappa-containing antibody production to some bacterial polysaccharides and perhaps to other antigens.**

## Introduction

*Haemophilus influenzae* type b (Hib),[1] pneumococci, and meningococci remain the major causes of serious bacterial infections in young children. Antibodies to the polysaccharide capsules of these pathogens have been shown to be important in protection against infection (1–4). The response to polysaccharide antigens is thought to be predominantly the IgG2 subclass, and we have previously demonstrated that the human antibody response to bacterial polysaccharide antigens is correlated with the individual's total IgG2 subclass concentration (5). In addition, several lines of evidence suggest that genetic factors influence the human antibody response to these antigens and susceptibility to infection by these pathogens (6–10).

Allotypes are genetic markers expressed as antigens on the constant regions of heavy or light chains of immunoglobulins. We have recently reported that the allotype of IgG-2 heavy chains, G2m(n), is correlated with the human IgG antibody response and susceptibility to polysaccharide encapsulated bacteria in Caucasians (11). Here we examine the relationship of Km allotypes, antigenic markers on kappa light chains, to the light chain distribution of the antibody response.

Human kappa light chain allotypes are designated Km(1), Km(2), and Km(3). Km(2) is almost always found in association with Km(1). Usually Km(1) and Km(3) behave as antithetical alleles. Because the Km allotypes are expressed on light chains they occur on molecules of all isotypes (IgG, IgM, IgA, IgD, and IgE). The Km loci are located on chromosome 2 and thus are unlinked to the HLA loci (12). All allotypes are inherited in a Mendelian fashion and are co-dominant. In Caucasians, Km(1) occurs in ~10–18% of individuals.

We hypothesized that any association between Km allotypes and antibody response might be confined to antibody containing kappa light chains and be present for every immunoglobulin isotype. We therefore examined the relationship of Km allotype to total kappa and lambda antibody concentrations as well as to the heavy and light chain specific antibody to three polysaccharide antigens in 129 immunized caucasian adults. We chose the capsular polysaccharide of Hib, *Streptococcus pneumoniae* type 3, and *Neisseria meningitidis* group C. The first two had previously been demonstrated to elicit a kappa-predominant and lambda-predominant response, respectively (13). *N. meningitidis* group C was examined as a second antigen with a kappa-predominant response.

## Methods

### *Donors*

57 male and 72 female Caucasian plasma donors with a mean age of 33 yr were immunized with 0.5 $cm^3$ 14-valent pneumococcal vaccine (Pneumovax, lot 1912B; Merck, Sharp and Dohme, West Point, PA) and concurrently with 0.5 $cm^3$ Hib vaccine (lot 764-CF320; Merck, Sharp and Dohme) and 0.5 $cm^3$ bivalent meningococcal vaccine (Menomune A/C; Connaught Laboratories, Swiftwater, PA), which were combined in the same syringe. Serum for immunoglobulin allotyping, immunoglobulin concentrations, and polysaccharide antibodies was obtained before and 4 wk after immunization and stored at −20°C until assay.

### *Assays*

Antibody to the capsular polysaccharide of Hib was measured by radioimmunoassay (RIA) (14), 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). Pneumococcal and meningococcal antibody was quantitated by RIA using radiolabeled pneumococcal type 3 antigen in Dr. G. Schiffman's laboratory (15) and meningococcal group C antigen in Dr. E. Gotschlich's laboratory (16).

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1. *Abbreviation used in this paper:* Hib, *Haemophilus influenzae* type b.

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IgG, IgM, and IgA antibody to the capsular polysaccharides of Hib and meningococcus group C were quantitated by enzyme-linked immunosorbent assay (ELISA) using the respective antigens coupled to tyramine with cyanogen bromide as previously described (11). Light chain specific anti-polysaccharide antibody was determined by an adaptation of this ELISA procedure using goat antihuman kappa and lambda alkaline phosphatase conjugates (Tago, Burlingame, CA).

Total kappa and lambda concentrations were determined by capturing immunoglobulin with goat anti-kappa or lambda IgG fractions (Atlantic Antibodies, Scarborough, ME) adsorbed to microtiter plates at 1 μg/ml and developing with goat anti-human kappa and lambda alkaline phosphatase conjugates (Tago).

The method of Zollinger and Boslego (17) was employed to estimate the concentrations of specific antibody in a human hyperimmune plasma pool (18) as a reference for ELISAs. Affinity-purified human IgG, IgM, or IgA fractions (Cappel Laboratories, Cochranville, PA) or myeloma proteins of known light chain composition (kindly supplied by Dr. Peter Schur, Brigham & Women's Hospital, Boston, MA) were used as standards. Zollinger and Boslego (17) showed that for anti-meningococcal polysaccharide antibody concentrations, this method of standardization corresponded well with estimates obtained by the quantitative precipitin method. However, because the assays may theoretically be affected by the antibody binding specificity or other properties of the capturing and developing antibodies, we have elected to express the antibody concentration in ELISA units rather than nanograms per milliliter.

Km immunoglobulin allotypes were determined by hemagglutination inhibition in the laboratory of G. de Lange (van Loghem, reference 12).

### *Statistical methods*

Data organization and analysis were performed on the PROPHET system, a national computer system sponsored by the Chemical/Biological Information Handling Program of the National Institutes of Health. All antibody concentrations were converted to logarithms to normalize their distributions before calculating means (19). The two-tailed *t* test was used to compare means. Correlations and multiple linear regression analyses were performed with the logarithms of the antibody concentrations.

## Results

### *Light chain composition*

The concentrations of antibody containing kappa or lambda light chains were determined in the 129 Caucasian adults after immunization with polysaccharide antigens. The geometric mean ratio of kappa light chains to lambda light chains in total immunoglobulin was 1.9. This kappa predominance in total immunoglobulin has also been noted by other methods (20). However, the kappa/lambda ratio for specific anti-polysaccharide antibody differed (Table I).

The geometric mean kappa/lambda ratio of antibody to the capsular polysaccharide of Hib and *N. meningitidis* group C were increased at 2.8 and 4.9, respectively. In contrast, the kappa/lambda ratio of *S. pneumoniae* type 3 was lower at 0.50.

### *Km(1) allotype and heavy chain specific response*

The frequency of the Km(1) allotype was 11% (14:129) in our study population, which is similar to other Caucasian populations studied (21). 2 individuals were homozygous for Km(1) and 12 were heterozygous. We first examined the total and heavy chain specific response to the three polysaccharide antigens in the 129 patients.

The 14 Km(1)-positive individuals had lower total concentrations of antibody to Hib and *N. meningitidis* group C polysaccharide than the 115 Km(1) negatives (Table II). We then examined the heavy chain specific (class) response to Hib and *N. meningitidis* group C polysaccharide of Km(1) positive and negative individuals. Since these two groups did not differ in their total binding antibody to *S. pneumoniae* type 3, we did not examine the heavy chain specific response for this antigen. The Km(1) positives had lower concentrations of IgG, IgM, and IgA antibody to both polysaccharide antigens compared with the 115 negative individuals (Table II). The differences were significant for IgG ($P = 0.007$) and IgA ($P = 0.023$) to Hib and IgM ($P = 0.003$) to *N. meningitidis* group C.

*Table I. Light Chain Distribution of Immunoglobulin and of Specific Antibody after Immunization with Bacterial Polysaccharides in 129 Adults*

| | Geometric mean antibody concentrations (ELISA units) | | |
|---|---|---|---|
| | Kappa | Lambda | Ratio* |
| Total immunoglobulin ($\times 10^3$) | 14,400 | 7,700 | 1.87 |
| Antibody to: | | | |
| *H. influenzae* type b | 10,700 | 3,820 | 2.80 |
| *N. meningitidis* group C | 15,600 | 3,160 | 4.94 |
| *S. pneumoniae* type 3 | 3,770 | 7,594 | 0.50 |

* Kappa ÷ lambda.

*Table II. Relationship between the Km(1) Allotype and Heavy Chain Specific Antibody Concentrations after Immunization with Polysaccharide Antigens*

| | Geometric mean antibody concentration after immunization | | |
|---|---|---|---|
| Antibody to: | Km(1)+ | Km(1)− | *P* value* |
| Assay units | *n* = 14 | *n* = 115 | |
| *H. influenzae* type b | | | |
| IgG (*ELISA units*) | 3,580 (1,360–9,431)‡ | 11,400 (8,730–14,900) | 0.007 |
| IgM (*ELISA units*) | 808 (350–1,867) | 1,430 (1,110–1,840) | 0.146 |
| IgA (*ELISA units*) | 705 (429–1,160) | 1,550 (1,235–1,950) | 0.023 |
| Total (*RIA ng/ml*) | 18,900 (11,300–31,400) | 31,700 (25,400–39,600) | 0.121 |
| *N. meningitidis* group C | | | |
| IgG (*ELISA units*) | 3,800 (2,010–7,190) | 7,900 (5,830–10,700) | 0.348 |
| IgM (*ELISA units*) | 1,160 (901–1,490) | 1,850 (1,670–2,050) | 0.003 |
| IgA (*ELISA units*) | 389 (213–710) | 708 (547–917) | 0.057 |
| Total (*RIA ng/ml*) | 21,900 (15,300–31,300) | 32,500 (28,300–36,100) | 0.045 |
| *S. pneumoniae* type 3 | | | |
| Total (*RIA ng/ml*) | 10,400 (6,840–15,900) | 10,500 (8,680–12,800) | 0.625 |

* A two-tailed *t* test was used for normally distributed values and a Mann-Whitney test for nonnormally distributed values. ‡ 95% confidence interval of the mean.

### *Km(1) allotype and light chain specific response*

We next examined the light chain specific antibody concentrations after immunization with polysaccharide antigens in these 129 adults. The 14 Km(1)-positive individuals had lower concentrations of kappa-containing anti-polysaccharide antibody for Hib ($P = 0.0029$) and *N. meningitidis* group C ($P = 0.003$) than the 115 Km(1) negatives. For the antigen that elicits a lambda predominant response, *S. pneumoniae* type 3, there were no differences between the groups. Km(1)-positive individuals did not differ statistically from Km(1) negatives in their lambda anti-polysaccharide concentrations to any of the three polysaccharides.

To determine if the Km(1) association was specific for antibody to polysaccharides we examined the relationship of Km(1) to total kappa and lambda antibody. The Km(1) positives had a lower geometric mean kappa antibody concentration of 12,800 $10^3$ ELISA units as compared with 14,500 $\times$ $10^3$ ELISA units ($P = 0.079$) (Table III). The presence of the Km(1) allotype did not significantly correlate with total lambda antibody concentrations.

### *Km(1) and G2m(n) allotype*

Since we had previously demonstrated an association of a heavy chain allotype marker [G2m(n)] and IgG antibody response to polysaccharides (11), we performed a multiple linear regression analysis to examine the independent nature of the Km(1) and G2m(n) associations (Table IV). The significant correlations of Km(1) with decreased antibody to Hib of the IgG ($P = 0.006$) and IgA classes ($P = 0.028$) and of the kappa light chain type ($P = 0.037$) was confirmed in this analysis. Even when the effect of G2m(n) on IgG antibody was removed, Km(1) remained significantly correlated with decreased IgG class antibody. For antibody to *N. meningitidis* group C, Km(1) was significantly and independently correlated with decreased antibody of the IgM class ($P = 0.003$) and kappa chain type ($P = 0.003$).

*Table III. Relationship between Km(1) Allotype and Light Chain Specific Antibody Concentrations after Immunization with Polysaccharide Antigens*

| | Geometric mean antibody concentration (ELISA units) after immunization | | |
|---|---|---|---|
| | Km(1)+ ($n = 14$) | Km(1)− ($n = 115$) | *P* value* |
| *H. influenzae* type b | | | |
| Kappa | 5,320 (2,710–10,500)‡ | 11,600 (9,300–14,500) | 0.029 |
| Lambda | 7,074 (4,520–11,100) | 3,540 (2,700–4,640) | |
| *N. meningitidis* group C | | | |
| Kappa | 8,280 (5,550–12,400) | 16,200 (14,200–18,500) | 0.003 |
| Lambda | 2,260 (1,230–4,150) | 3,290 (2,740–3,950) | |
| *S. pneumoniae* type 3 | | | |
| Kappa | 3,980 (2,660–5,950) | 3,750 (3,230–4,356) | |
| Lambda | 5,950 (3,910–9,040) | 7,830 (6,820–8,990) | |
| Total antibody ($\times 10^3$) | | | |
| Kappa | 12,800 (11,200–14,600) | 14,500 (13,900–15,200) | 0.079 |
| Lambda | 7,300 (6,050–8,880) | 7,800 (7,400–8,220) | |

* A two-tailed *t* test was used for normally distributed values and a Mann-Whitney test for nonnormally distributed values.
‡ 95% confidence interval of the mean.

*Table IV. Correlation of G2m(n) and Km(1) Allotypes with Anti-polysaccharide Antibody Concentrations after Immunization*

| | Partial correlation coefficients* (*P* value) for immunoglobulin allotypes | |
|---|---|---|
| Antibodies to (heavy or light chain): | G2m(n) | Km(1) |
| *H. influenzae* type b | | |
| IgG | +0.26 (0.003) | −0.25 (0.006) |
| IgM | +0.06 | −0.12 |
| IgA | −0.00 | −0.20 (0.028) |
| Kappa | +0.04 | −0.19 (0.037) |
| Lambda | −0.04 | +0.15 |
| *N. meningitidis* group C | | |
| IgG | +0.22 (0.014) | −0.15 |
| IgM | −0.08 | −0.47 (0.003) |
| IgA | +0.00 | −0.13 |
| Kappa | −0.04 | −0.28 (0.003) |
| Lambda | +0.07 | −0.15 |

* Partial coefficients were obtained by multiple linear regression analysis. The coefficients given are calculated with the significant variables included in the regression model.

## Discussion

We had previously reported a correlation between G2m(n), an antigenic marker on the heavy chain of IgG2 subclass immunoglobulins, and the IgG antibody concentrations to bacterial polysaccharide antigens in Caucasians (11). In addition we documented a significant correlation between the low-responding G2m(n) negative phenotype and risk of Hib infection in young Caucasian children. Our present study examined the relationship of Km(1), an antigenic marker of kappa light chains, to anti-polysaccharide antibody after immunization. We hypothesized that since the G2m(n) association was confined to IgG antibody, the Km(1) association might be confined to kappa antibody and would therefore not be class specific.

The present data demonstrated a correlation of Km(1) with decreased concentrations of antibody of the kappa type for two of three polysaccharides examined. This correlation was significant for Hib and *N. meningitidis* group C, both of which elicited a predominantly kappa response. In addition, the correlation was seen for IgG, IgM, and IgA class antibody. The Km(1) allotype was also associated with a decreased concentration of kappa containing immunoglobulin ($P = 0.079$).

Others have noted correlations between Km(1) allotype and anti-polysaccharide responses. Pandey et al. reported that Caucasian children with the Km(1) allotype had higher antibody responses to group C meningococcal vaccine but lower responses to Hib vaccine than Km(1) negative children (22). In direct con-

trast to our findings, Granoff et al. noted that both Caucasian and Black children with the Km(1) marker had higher responses to immunization with Hib-pertussis complex vaccine than children lacking Km(1) (23). However, this correlation was noted only for IgG class and not for IgM class antibody. The light chain specific response was not examined. In addition, Blacks lacking the marker were at increased risk for Hib disease although Whites were not. The differences between these studies may relate to the type of vaccine used to stimulate antibody responses and to differences in age, race, or genetic make-up of the study populations.

Our study did not examine the mechanism of the Km(1)-associated influence on anti-polysaccharide antibody production. However, our findings indicate that the Km(1)-associated regulatory influence is confined to kappa chains and is probably not restricted to polysaccharide antigens. The Km(1) allotype correlation reached significance only for the antigens that elicited a predominant kappa response, Hib and *N. meningitidis* group C. Other antigens (nonpolysaccharides) that elicit predominantly kappa antibody may have a similar association between response and Km(1) allotype. Specific mechanisms that could be responsible include decreased production or secretion of kappa chain containing antibody at the B cell level for Km(1) individuals. Alternatively, allotype- or idiotype-mediated regulation of T cell interactions with B cells displaying kappa antibody could be involved. This would result in a light chain–restricted yet not class restricted association as was observed in this study. In animals, T helper cells with specificity for allotypes, idiotypes, and isotypes have all been demonstrated (24–26). In mice, the allotype regulation of the antibody response to sheep erythrocytes has been demonstrated to be mediated through T cell help (27).

Allotype-associated regulatory influences on anti-polysaccharide antibody production may play an important role in determining disease susceptibility as well as the protective efficacy of new bacterial vaccines. Of relevance, in this regard, is the observation that young children have lower kappa/lambda ratios than adults (20). Thus, an allotype-associated impairment in the kappa antibody response may be of particular significance for this age group and for antigens that elicit predominantly kappa antibody such as Hib. The effect of age on light chain specific response, functional comparisons of kappa and lambda anti-polysaccharide antibody, and the mechanisms of Km(1) allotype–associated regulatory influence need to be examined further.

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