11-1-1981

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ABSTRACT Serum samples were collected from 120 healthy adult volunteers (105 Caucasians and 15 Negros) before and after immunization with meningococcal polysaccharide (MPS) group B vaccine. Antibodies to MPS group B were measured and sera were typed for several Gm and Km(1) allotypes. A significant association was found between the Km(1) allotype and immune response to MPS group B in Caucasians.

INTRODUCTION

Major histocompatability complex (MHC)-linked and immunoglobulin (Ig) allotype-linked immune response (Ir) genes have been extensively studied in experimental animals (1). Several recent observations by us and others suggest that such genes also exist in man (2–4). The data presented here indicate the existence of an allotype-linked Ir gene(s) that regulates the humoral immune response to meningococcal polysaccharide (MPS) group B.

Group B *Neisseria meningitidis* continues to be responsible for a major proportion of meningococcal disease in the United States, Europe and England (5). Protection from meningococcal disease has been correlated with the presence of serum bactericidal antibodies (6). Individuals who are "nonresponders" may thus be at an increased risk of developing group B meningococcal disease. Our findings suggest genetically determined differences in the magnitude of humoral immune responses to this organism, which might influence disease susceptibility.

Publication no. 465 from the Department of Basic and Clinical Immunology and Microbiology, Medical University of South Carolina. Research supported in part by U. S. Public Health Service grants HD-09938 and CA-25746.

Received for publication 22 June 1981 and in revised form 25 August 1981.

[2] *Abbreviations used in this paper*: Ir, immune response; MHC, major histocompatibility complex; MPS, meningococcal polysaccharide.

METHODS

Subjects and vaccines. A total of 120 healthy adult male volunteers (105 Caucasians and 15 Negroes), 18–21 yr of age, were vaccinated with meningococcal group B vaccine (120 μg in 0.5 ml of saline), either lot BP2-WZ-2 or lot BP2-4. The two lots of vaccine are of similar composition and antigenicity as determined by in vitro tests. Both are approximately a 1:1 noncovalent complex of group B polysaccharide and outer membrane protein. Blood was collected immediately before and 2 wk after immunization.

Antibody determination. Antibodies to MPS group B were determined by a radioactive antigen binding assay. This assay uses group B polysaccharide intrinsically labeled with tritium at a sp act of ~200–500 cpm/ng. Chlorine-36 as Cl^- was added as a volume maker. After incubation of antigen and antiserum at 4°C overnight, an equal volume of cold saturated ammonium sulfate was added and the sample was centrifuged to pellet the precipitate. A sample of the supernatant was counted and calculations were made as described by Gotschlich et al. (7). As a standard, a relatively high titered human serum (58 μg antibody per milliliter as determined by quantitative precipitin assay) was arbitrarily assigned a value of 100 U of antibody per milliliter.

Ig allotyping. Our standard hemagglutination inhibition technique (8) was used to detect Gm and Km(1) markers, the hereditary antigenic determinants on the constant regions of γ chains and κ-type light chains, respectively. Serum samples were typed for Gm antigens 1, 2, 3, 5, 6, 13, 14, 17 and 21, as well as Km(1).

Statistical analysis. Correlation coefficients were calculated to determine whether there was an association between preimmunization antibody levels and the postimmunization increase in antibody titer, and analysis of variance was used to determine whether there was any association between Gm or Km(1) phenotypes and the immune response to MPS group B. All computations were performed using University of California at Los Angeles computer program BMDP2V: Analysis of Variance and Covariance.

RESULTS

In contrast to MPS groups A and C, for which a significant negative correlation has been reported between preimmunization titer and titer increase (9), we found

TABLE I

Mean Immune Response to Meningococcal Group B Polysaccharide in Relation to Gm and Km(1) Phenotypes in Caucasians

Phenotype	No.	Mean response*
Gm 1, 17; 21	4	19.25±9.18
Gm 1, 2, 17; 21	10	2.70±2.45
Gm 3; 5, 13, 14	48	13.44±1.48
Gm 1, 3, 17; 5, 13, 14, 21	32	11.53±3.21
Gm 1, 2, 3, 17; 5, 13, 14, 21	11	11.18±4.53
Km(1)+	18	18.22±3.52‡
Km(1)−	87	10.49±1.44

* Mean immune response is difference between postimmunization and preimmunization antibody levels in units of anti-MPS B per milliliter of serum.
‡ $F = 4.77$; $P = 0.031$.

no significant correlation between preimmunization antibody levels and immune response to MPS group B.

The mean immune responses to MPS group B in Caucasian recipients with various Gm and Km(1) phenotypes are shown in Table I. No significant associations were found between any of the Gm phenotypes and immune response to MPS group B; however, a significant association was found between Km(1) and immune response to MPS group B ($F = 4.77$, $P = 0.031$). The mean preimmunization levels of antibody to MPS group B for subjects positive for Km(1) and those without this allotype were approximately the same (7.67 vs. 7.56 U/ml of serum). After immunization, however, the immune response was dramatically higher in Km(1) positive than in Km(1) negative individuals (18.22 vs. 10.49 U/ml of serum). The significance of the association between Km(1) and immune response to MPS group B in Negros (Table II) was borderline ($P = 0.077$); however, because of the small number of Negro subjects in the study, no definite conclusions can be drawn with regard to this group.

DISCUSSION

The significant association between Km(1) and immune response to MPS group B in Caucasians is noteworthy. In an earlier study (2), we reported a significant association between Km(1) and the response to MPS group C in Caucasian infants 18–20 mo old. Two probable explanations can be forwarded to account for these results. First, the Km(1) allotype itself may regulate the immune responses to MPS group B and C. Several studies have shown significant correlations between Gm phenotypes and serum concentrations of IgG subclasses, as well as between Gm phenotype and antibody activity (10). No such studies have been undertaken with respect to Km allotypes. Km, like Gm, may also influence Ig concentrations and antibody activity because it is present in all Ig classes, not merely IgG. A second explanation may be that there exist in man Ir genes that regulate the immune responses to MPS groups B and C, and that alleles of these genes are in linkage disequilibrium with the Km alleles. Although conclusive evidence for the existence of Ir genes can be obtained only after family studies (which are difficult to undertake in humans), the data presented here are suggestive of such genes in man.

Bactericidal antibodies induced by MPS group C are predominantly IgG (11), and Siber et al. (12) have recently reported a correlation between serum IgG_2 concentrations and the antibody response to several other bacterial polysaccharide antigens. In contrast, the antibodies induced by MPS group B are IgM (5). Involvement of Km(1) in immune responses to both MPS groups B and C can be explained by association of the κ chain with both γ and μ chains.

One way to corroborate the results reported here would be to study individuals who were immunized with MPS group B and nevertheless developed meningococcal meningitis. Our results would predict such people to be predominantly Km(1) negative. Such studies are currently in progress in our laboratory.

In future studies of the kind reported here, it would be interesting to study the same group of individuals for both Ig allotypes and HLA antigens. It has been shown that HLA- and Ig allotype-linked genes interact in determining levels of serum antibodies to the bacterial antigen monomeric flagellin (3), and a similar situation may exist for other immunogens. Such interactions between polymorphic genes may provide not only a mechanism for increasing the specificity of antibody reactions but also a more flexible means of evolutionary adaptation to newly encountered and potentially harmful infections (3). Further studies of immunogenetic markers and antibody responses may also shed some light on the selective mechanism that maintains HLA, Gm, and Km polymorphisms; indeed, associations between these antigenic determinants and specific antibody responses may be the selective force

TABLE II

Mean Immune Response to Meningococcal Group B Polysaccharide in Relation to Gm and Km(1) Phenotypes in Negroes

Phenotype	No.	Mean response
Gm 1, 17; 5, 13, 14	8	17.12±6.38
Gm 1, 17; 5, 6, 13, 14	4	9.00±1.96
Gm 1, 3, 17; 5, 13, 14	3	0.67±8.41
Km(1)+	8	18.25±5.33*
Km(1)−	7	4.14±4.99

* $F = 3.66$; $P = 0.077$.

maintaining their polymorphism. In other words, selection may operate through Ir genes, with the HLA antigens and Ig allotypes serving merely as markers.

ACKNOWLEDGMENTS

We thank Carl Lowder, Jr., for technical assistance and Charles L. Smith for editorial assistance.

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