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Research Article

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Immunoglobulin κ Chain Allotypes (KM) in Onchocerciasis

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Abstract

GM and KM allotypes, powerful tools for genetic characterization of human populations, have been shown to play an important role in genetic predisposition to some infectious diseases. Two diverse racial groups—Afro-Ecuadorians and Amerindians—living in a single restricted geographical area of Ecuador, appear to have different risk factors for acquisition and clinical expression of onchocerciasis, a disease caused by the filarial parasite *Onchocerca volvulus*. In this study, GM and KM allotypes were determined in 25 Afro-Ecuadorians and 24 Amerindians infected with *Onchocerca volvulus* (INF) and in putative immune individuals (PI). In Afro-Ecuadorians, the frequency of the homozygous KM 3 phenotype was significantly decreased in INF as compared with the PI group (20 vs. 68%; $P = 0.0012$), while the frequency of the heterozygous KM 1,3 phenotype was increased in INF as compared with the PI subjects (48 vs 9%; $P = 0.0044$). These results suggest that in Afro-Ecuadorians KM 3 is associated with a lower relative risk (resistance), whereas KM 1,3 is associated with an increased risk (susceptibility) of onchocerciasis. (*J. Clin. Invest.* 1995. 96:2732–2734.) Key words: GM • immunity • phenotype • haplotype • gene frequency

Introduction

For most polymorphic systems, the differences among human populations are quantitative rather than qualitative. The GM allotypes—hereditary antigenic determinants of IgG heavy chains located on chromosome 14—are unique in the sense that every major race is characterized by its own array of GM haplotypes (1–3). For this reason, the GM system has long been recognized a useful genetic marker for classification of human populations. The KM allotypes—hereditary antigenic determinants of k type light chains—are inherited via three alleles on chromosome 2. All populations studied are polymorphic for KM allotypes.

The biological role and the evolutionary reasons for GM and KM polymorphisms remain unknown. The marked differences in the distribution of these determinants among different ethnic groups raise questions concerning the nature of the selective mechanism that maintains these differences. Associations between GM and KM allotypes and specific antibody responses could be a likely selective force (4). To test this hypothesis, immunoglobulin allotypes have been intensively studied for their role in susceptibility/resistance to several immunologically mediated diseases. Numerous disease associations have been reported, including some with infectious diseases (5–10).

The difference in the clinical expression of onchocerciasis between Afro-Ecuadorians and the Amerindians (11–13), and its association with the HLA complex (14, 15), suggest the involvement of genetic factors in susceptibility to this infection. In addition, the local and systemic immune mechanisms are clearly implicated in the *Onchocerca*-induced tissue damage (16). The mediation by the immune and genetic factors in the etiology of onchocerciasis provided us with a good rationale to examine the role of the genetic markers of immunoglobulins in susceptibility/resistance to this disease. The aims of the present study were to determine whether there are significant differences in the distribution of GM and KM phenotypes in subjects infected with *Onchocerca volvulus* (INF)[1] and putatively immune (PI) individuals, and if such differences are ethnically restricted. A group of individuals have been identified in a hyperendemic focus of onchocerciasis in Ecuador, who have been rigorously characterized as having been infection-free for the past 13 years and therefore classified as putatively immune (17). These individuals have been shown to have significantly different *Onchocerca volvulus*-specific humoral and cellular immune responses from those infected with this parasite (18).

Methods

Study population. The individuals included in the study had all lived within the endemic area for at least 17 yr as previously described (17). Each had been evaluated for onchocerciasis four times over the previous 13 yr. These evaluations included a physical examination, medical history, nodule palpation, four skin snips, and a complete ophthalmologic evaluation.

The INF group consisted of 24 indigenous Amerindians and 25 Afro-Ecuadorians living in the hyperendemic area of Esmeraldas Province of Ecuador, who were all microfilademic before and at the time of the study. The PI group consisted of 16 Amerindians and 22 Afro-Ecuadorians who were negative for onchocerciasis at the time of the study and for the previous 13 yr.

60 ml of blood was drawn from each individual and separated on a Ficoll-diatrizoate gradient. The plasma and mononuclear cells were stored in liquid nitrogen.

GM and KM allotyping. Plasma samples were typed for G1M 1,2,3,17, G2M 23, G3M 5,6,13,21 and KM 1,3 by a standard hemagglutination-inhibition method (19, 20). These markers are sufficient to

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1. *Abbreviations used in this paper:* INF, subjects infected with *Onchocerca volvulus*; PI, putatively immune.

Table I. Distribution of GM and KM‡ Phenotypes in Afro-Ecuadorians Infected with Onchocerca volvulus (INF) and in Putative Immune (PI) Individuals*

Phenotype	INF (n = 25) No.	PI (n = 22) No.
	%	%
GM 1,17 5,13	17 (68)	16 (73)
GM 1,17 5,6,13	4 (16)	2 (9)
GM 1,17 5,13,21	2 (8)	3 (14)
GM 1,17 21	1 (4)	0
GM 1,2,17 5,13,21	1 (4)	1 (5)
KM 1	8 (32)	5 (23)
KM 3	5 (20)	15 (68)
KM 1,3	12 (48)	2 (9)

* Fisher's Exact Test (2-Tail), P = 0.913; ‡ Fisher's Exact Test (2-Tail), P = 0.0015.

detect the haplotypes commonly present in all major races. The notation follows the international system for human gene nomenclature (21). Briefly, the method uses human blood group ORh + erythrocytes coated with anti-Rh antibodies of known GM and KM allotypes and a panel of monospecific anti-allotype sera. Test sera containing immunoglobulin of particular allotype inhibit hemagglutination of anti-allotype antibody, whereas negative sera do not.

Statistical analysis. Fisher's two-tail exact test (22) was used to determine the significance of differences in GM and KM phenotype distributions between the INF and PI groups, employing the Statistical Analysis Software (SAS Institute Inc., Cary, NC). Bonferroni's correction for type I error rates was used to adjust for the number of comparisons made.

Results

The distribution of GM and KM phenotypes in Afro-Ecuadorian subjects is given in Table I. The majority of the INF and PI subjects had typical Negroid GM phenotypes, which can be explained by postulating two common haplotypes—GM* 1,17 5,6 and GM* 1,17 5,13. Presence of GM 21 in three phenotypes indicates admixture with Amerindian haplotypes—GM* 1,17 21 and GM* 1,2,17 21. The parents of one patient with GM 1,17 21 were probably heterozygous for a Negroid and the Amerindian haplotype GM* 1,17 21. Such genetic admixture in Ecuadorian populations has been reported previously (23). The distribution of GM phenotypes in INF and PI groups was not significantly different (P = 0.913).

Fisher's exact test (two-tail), considering all KM phenotypes, shows that there is a significant difference in the distribution of various phenotypes between the INF and PI groups (P = 0.0015). Further dissection of this association elucidates that the discrepancy in the distribution of KM 3 and KM 1,3 contributes the most to the total variation. The associations with these two phenotypes, however, were in opposite directions; the frequency of KM 3 was significantly decreased in INF as compared with the PI group (20 vs 68%; P = 0.0012; OR = 0.12), while the frequency of KM 1,3 phenotype was increased in INF as compared with the PI individuals (48 vs 9%; P = 0.0044; OR = 9.23). (These results are significant even after the most conservative correction for the experimentwise error rate.) No significant difference was found in the frequency of KM 1 between the INF and PI groups (P = 0.530).

Table II. Distribution of GM and KM‡ Phenotypes in Amerindians Infected with Onchocerca volvulus (INF) and in Putative Immune (PI) Individuals*

Phenotype	INF (n = 24) No.	PI (n = 16) No.
	%	%
GM 1,17 5,13,21	1 (4)	0
GM 1,17 21	20 (83)	15 (94)
GM 1,2,17 21	3 (12)	1 (6)
KM 1	2 (8)	3 (19)
KM 3	11 (46)	9 (56)
KM 1,3	11 (46)	4 (25)

* Fisher's Exact Test (2-Tail), P = 0.779; ‡ Fisher's Exact Test (2-Tail), P = 0.283.

Table II shows the distribution of GM and KM phenotypes in the Amerindians. With the exception of one patient, all subjects can be explained by postulating the segregation of common Amerindian haplotypes—GM* 1,17 21 and GM* 1,2,17 21. The one patient with the GM 1,17 5,13,21 phenotype is probably heterozygous for the Negroid haplotype GM 1,17 5,13 and the Amerindian haplotype GM* 1,17 21. Neither GM or KM phenotypes in this population was significantly associated with onchocerciasis infection (P = 0.779 and 0.283, respectively).

Discussion

The distribution of KM alleles in our subjects is comparable to those reported in several other larger population studies (2). The results presented here indicate a distinct association between putative immunity to onchocerciasis and the possession of the KM 3 phenotype and between infection and the KM 1,3 phenotype in the Afro-Ecuadorian population in the Ecuadorian focus. The risk of onchocerciasis infection in Afro-Ecuadorian individuals with KM 3 was almost 90% less than the risk for those with KM 1 or KM 1,3 (OR = 0.12). Conversely, the risk of disease in the Afro-Ecuadorian population is over nine times greater among those with KM 1,3 compared with those with KM 1 or KM 3 (OR = 9.23). There are at least two possible explanations for these associations.

The KM locus could itself affect susceptibility/resistance to onchocerciasis, possibly through its effect on cellular and humoral immunity to *Onchocerca volvulus*. These determinants have been shown to be associated with immunity to other microbial agents (24–30). The lower relative risk of onchocerciasis in KM 3 subjects may be the result of a more vigorous immune response to *Onchocerca volvulus* in these subjects as compared with those who are KM 1 or KM 1,3. Perhaps the immunoglobulin molecules bound to the surface of B lymphocytes in individuals homozygous for the KM 3 allotype act as more compatible receptors for the antigens of *Onchocerca volvulus*—and thus provoke a strong humoral immunity, whereas the immunoglobulin molecules of the KM 1,3 heterozygous individuals form a less compatible receptor for the critical epitopes of this pathogen. A much larger study population will be needed to conclu-

sively determine the effect of KM allotypes on immune responsiveness to *Onchocerca volvulus*.

Alternatively, there may be another major locus for susceptibility to onchocerciasis on chromosome 2, distinct from KM, whose alleles are in significant linkage disequilibrium with those of the KM locus. This putative linkage disequilibrium may give rise to the associations observed. The data reported here cannot distinguish between the two mechanisms. The distinction between the two possibilities, however, is important, as it has important implications in understanding the host factors influencing pathogenesis of onchocerciasis. Perhaps linkage studies—particularly partitioned association-linkage approach—involving large families with onchocerciasis may be able to resolve this issue (31–32).

The racial differences in the KM allotype associations observed in this study are reminiscent of a study involving black and white children with *Haemophilus* meningitis: the KM 1 allotype was found to be associated with a lower relative risk of the disease in black children, but the risk of meningitis was not decreased in the white children with the KM 1 allotype (27). The KM association with onchocerciasis in the Afro-Ecuadorians may be a result of its interaction with another genetic marker whose frequency is different in blacks and Amerindians—possibly a gene of the HLA system, a host factor previously implicated in onchocercal infections (14, 15). It is relevant to note here that immunoglobulin allotypes and HLA antigens have been shown to interact to cause susceptibility to several diseases (5). Simultaneous examination of immunoglobulin allotypes, HLA antigens and other relevant polymorphic systems, e.g., the T cell receptor, TNF-α, may shed light on the mechanisms responsible for the racial differences in the associations reported here.

To our knowledge, this is the first report implicating immunoglobulin allotypes in susceptibility to onchocerciasis.

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