

Influence of Host Genetics on the Severity of Coccidioidomycosis

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Coccidioidomycosis, a mild flulike illness in approximately 40% of infected persons, progresses to severe pulmonary or disseminated disease in 1% to 10% of symptomatic cases. We examined host genetic influences on disease severity among class II HLA loci and the ABO blood group. Participants included African-American, Caucasian, and Hispanic persons with mild or severe disseminated coccidioidomycosis from Kern County, California. Among Hispanics, predisposition to symptomatic disease and severe disseminated disease is associated with blood types A and B, respectively. The HLA class II DRB1*1301 allele marks a pre-disposition to severe disseminated disease in each of the three groups. Reduced risk for severe disease is associated with DRB1*0301-DQB1*0201 among Caucasians and Hispanics and with DRB1*1501-DQB1*0602 among African-Americans. These data support the hypothesis that host genes, in particular HLA class II and the ABO blood group, influence susceptibility to severe coccidioidomycosis.

Coccidioidomycosis (CM), also known as Valley fever, is caused by the dimorphic fungus *Coccidioides immitis*, endemic to the southwestern United States, Mexico, and Central and South America. In California before 1991, approximately 400 to 500 new cases were reported annually. During 1991 to 1994, the number of cases increased dramatically, with 1,200 new cases reported in 1991, 4,541 in 1992, and 4,137 in 1993 (1-4).

C. immitis is a soil-dwelling organism that blooms after the rainy season. Infection usually occurs in the dry season, when arthroconidia become airborne and can be inhaled by the host. In California in 1991, increased rainfall following a drought allowed increased fungal proliferation, resulting in an increased rate of infection. The 1994 Northridge earthquake led to an increase in infections associated with

landslides and dust clouds that aerosolized arthroconidia. Increased risk for symptomatic CM was directly proportional to time spent in the dust cloud (5).

Of those infected, an estimated 60% are asymptomatic, with the only evidence of exposure being delayed-type hypersensitivity (DTH) reaction to a coccidioidal antigen skin test. Of the symptomatic patients, 90% to 99% experience only mild flulike symptoms. More severe chronic pulmonary disease or disseminated infection beyond the thoracic cavity occurs in 1% to 10% of symptomatic cases, depending on ethnicity (N. Ampel, pers. comm.). Disseminated CM is particularly devastating and usually requires lifelong antifungal treatment. Risk factors include male sex, compromised immune status, pregnancy, diabetes, advancing age, and smoking (6-9).

Risk for disseminated CM seems to differ according to ethnicity (9). For example, during the 1977 windborne outbreak of CM in the nonendemic-disease region of Sacramento County, California, the rate per 100,000 of acute

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symptomatic pulmonary CM among African-American men compared with Caucasian men was 67 versus 19 (ratio 3.5:1) and of disseminated CM was 23.8 versus 2.5 (ratio 9.1:1). These differences could not be explained by differential exposure (11). More recently, in the endemic area of Kern County, California, African-American men had an adjusted odds ratio for disseminated CM of 28 (95% confidence interval [CI] 2-385), higher than that of any other ethnic group. No environmental or occupational exposures were associated with either severe pulmonary or disseminated disease (9). Both the range of response given *C. immitis* exposure and the apparent variation in susceptibility among ethnic groups suggest that genetic factors influence the development of symptomatic, severe, and disseminated CM.

T-cell-mediated immunity is important in the elimination of the fungus, but it eventually diminishes if the disease progresses (12,13). Although little is known about the role of T-cells in eliminating *C. immitis*, activated T-cells elicit a DTH inflammatory response, indicating a Th1-type response (14). While DTH reactivity is regulated by class II HLA interactions with T cells, the host immune response to intracellular pathogens is primarily regulated by class I HLA molecules. HLA genes are therefore prime candidates for the study of host genetic influences on the severity of CM. Some studies have shown disseminated CM to be associated with HLA antigens (A-9, B-5) and ABO blood group B (6,7,15,16). Both African-Americans and Filipinos have greater frequency of the B blood group and HLA-A9 than do Caucasians (16).

Kern County, where CM is highly endemic, contributed substantially to the 1991 to 1994 epidemic in California. We investigated the role of genetic factors in CM in a case-control study of persons from Kern County among patients with severe disseminated disease or mild disease. This study explored the possible association of HLA class II alleles and haplotypes and ABO phenotypes with severity of CM disease in three ethnic groups.

Methods and Study Design

Participants

Patients were recruited from the Kern County Health Department and Kern Medical Center. Severe cases (n = 109) were defined as

extrapulmonary disseminated CM, with disease spreading beyond the thoracic cavity, including prevalent and incident cases seen or diagnosed from 1995 through 1997. Mild cases (n = 83) were diagnosed during 1995 to 1996, with uncomplicated disease limited to the lung (or lymph nodes draining the lung) and not requiring hospitalization (Table 1). Incident cases, which had laboratory evidence of acute infection, were detected during a population-based surveillance study of CM in Kern County (11). All diagnoses were laboratory confirmed by serologic testing, enzyme immunoassay ([EIA], immunodiffusion, and complement fixation), or culture at the Kern County Health Department Laboratory.

For controls, data from ethnically and geographically matched populations were obtained from the literature (Table 1). For ABO phenotype comparisons, populations from San Francisco, were selected (17). Molecular data for comparing HLA allele frequencies were selected from healthy Caucasians from California (18), Hispanics from Los Angeles (19), and African-Americans from New York (20).

Table 1. Coccidioidomycosis participants, by ethnicity and severity of disease

Cases	Caucasians	African American			Total
		Hispanics	Caucasians	Other	
Severe	27	50	25	7	109
Mild	50	32	1	0	83
Total	77	82	26	7	192
Outside controls					
ABO	8,962	335	3,146		
HLA	107	115	241		
		(109 for DPB1)			

Genetic Testing

Standard ABO antigen blood typing was performed at the Kern County Health Department laboratories. All persons were typed for four HLA class II loci (DRB1, DQA1, DQB1, and DPB1) by molecular methods (21-24).

Statistical Analyses

For the HLA data, Hardy-Weinberg genotypic proportions were calculated to examine the equilibrium in each locus (25). Methods of testing genotypic ratios in the ABO system with the

recessive O allele were followed (26). HLA haplotype frequencies were estimated for each risk group by ethnicity and disease severity by using standard maximum likelihood methods (27,28).

ABO phenotype and allele frequencies and HLA class II allele and haplotype frequencies were compared by using 2 x k contingency tables that give an overall G-statistic, where k is the total number of alleles present in the comparison groups for a particular locus. All analyses were stratified by ethnicity. Comparing severe cases with mild cases assessed risk for disease progression after symptomatic infection, and comparing severe or mild cases with controls examined risk for disease severity and infection relative to each ethnic population. In any comparison, alleles present in fewer than three persons were pooled into a combined class of rare alleles. If the overall comparison showed statistically significant heterogeneity, the underlying difference was examined among individual alleles, phenotypes, or haplotypes by using the chi-square statistic, with odds ratios (OR) and Yates' corrected or Fisher's p values reported. Since only one African-American with mild disease was enrolled, analyses for African-Americans were limited to comparing severe cases with controls.

Interaction effects of ABO and HLA alleles associated with disease were assessed with the Mantel-Haenszel chi-square test and weighted OR by using EpiInfo version 6.01.

Results

Hardy-Weinberg Tests

Severe and mild cases were examined for deviations from Hardy-Weinberg equilibrium to validate the source populations and genotyping and to detect alleles that might be associated with disease. In each instance, the HLA loci DRB1 and DQA1 genotypic ratios were in equilibrium (Table 2). However, the DPB1 locus for mild cases among Hispanics and mild and severe cases among Caucasians and the DQB1 locus for Hispanics with mild cases were not in equilibrium ($p < 0.05$, Table 2). For DPB1 among Caucasians with mild cases, DPB1*0301-*0401 was less common than expected (none observed vs. three expected). For DPB1 for mild cases among Caucasians and Hispanics, genotypes with the *1401, *1601, and *4601 alleles were observed more often than expected. Other loci

Table 2. Hardy-Weinberg test results (p values) for HLA class II loci, by ethnicity and severity of disease^a

Cases	DRB1	DQA1	DQB1	DPB1	N
Hispanic, Severe	0.34	0.18	0.38	0.27	50
Hispanic, Mild	0.12	0.11	0.002	0.045	32
Caucasian, Severe	0.09	0.14	0.38	0.008	27
Caucasian, Mild	0.12	0.14	0.11	0.022	50
African-American, Severe	0.63	0.80	0.67	0.31	25

^aNumbers in bold indicate alleles not in Hardy-Weinberg equilibrium for a particular group and locus.

not in Hardy-Weinberg equilibrium ($p < 0.05$) had no significant deviations at individual genotypes.

ABO phenotype frequencies did not depart from Hardy-Weinberg equilibrium except for the large Caucasian control sample, which differed at $p = 0.04$, indicating a small deviation from equilibrium (data not shown).

Testing for Allelic Association

ABO blood types were analyzed by comparing the blood type phenotype to disease status (severe, mild, control) in contingency table tests. No differences between groups were seen for Caucasians ($p = 0.80$). This was also true for African-Americans when patients with severe disease were compared with controls ($p = 0.21$). Blood group phenotype frequencies among Hispanics, however, were highly heterogeneous with respect to disease status ($p < 0.001$) (Table 3). Among affected persons with either severe or mild disease, the A phenotypes were more frequent (OR = 1.71, $p = 0.18$ and OR = 5.53, $p < 10^{-4}$, respectively) and the B phenotypes less frequent (OR = 0.36, $p = 0.021$ and OR = 0, $p < 10^{-3}$, respectively) than among controls. Comparing severe versus mild cases among Hispanics, the A phenotypes were associated with decreased risk (OR = 0.31, $p = 0.024$) and the B

Table 3. Blood group antigen frequencies for coccidioidomycosis, by ethnicity and severity of disease

Ethnic group		Phenotype				N
		A	AB	B	O	
Hispanic ^a	Severe	0.24	0.02	0.12	0.62	50
	Mild	0.53	0.00	0.00	0.47	32
	Controls ^b	0.13	0.04	0.27	0.56	335
Caucasian	Severe	0.48	0.04	0.04	0.44	27
	Mild	0.46	0.02	0.12	0.40	50
	Controls ^b	0.41	0.03	0.11	0.45	8,962
African-American	Severe	0.12	0.04	0.16	0.68	25
	Controls ^b	0.28	0.04	0.19	0.50	1,540

^aABO phenotype distribution for cases and controls among Hispanics, $p < 0.001$

^bPopulation control frequencies from Mourant, 1976 (17)

phenotypes with increased risk (OR >5.2, Fisher's p = 0.039) of disseminated disease (Table 3).

Several of the HLA class II loci showed overall heterogeneity in the allele frequencies tested pairwise among the three disease status categories (Table 4). Effects occurred in all three ethnic groups. Significant effects occurred more extensively and with greater significance in

comparisons of cases versus controls than in comparisons of severe cases versus mild cases for DQB1, DRB1, and DR-DQ haplotype. This result might be due to the higher sample size in the controls, allowing greater power in comparisons with this group.

Significant HLA allelic and haplotypic associations differed for each ethnic group (Table 5). However, Caucasians and Hispanics share a haplotype, DRB1*0301-DQB1*0201, associated with reduced risk for disease. Among Caucasians, both mild and severe cases had this effect when compared with controls (OR = 0.2, p = 0.012 and OR = 0.1, p = 0.007, respectively). Among Hispanics, the effect was observed only for mild cases versus controls (OR = 0.1, p = 0.007).

The DRB1 allele, *1301, was associated with increased risk for disease in all three ethnic groups compared with controls. For Caucasians, mild cases were associated with this allele (OR = 2.6, p = 0.028), whereas severe cases were associated among Hispanics (OR = 4.9, p = 0.01) and African-Americans (OR = 4.2, p = 0.008). The DRB1-DQB1 haplotypes containing DRB1*1301 in each group were also associated with increased risk, although the DQB1 alleles in the haplotypes differed (Table 5).

Table 4. Significance levels from contingency table analyses of allele distributions for coccidioidomycosis, by ethnicity and severity of disease^a

Ethnic group	Locus	Severe	Severe	Mild
		vs. mild	vs. control	vs. control
Caucasians	DQA1	0.231	0.069	0.048
	DQB1	0.024	0.02	0.002
	DRB1	0.075	0.12	<0.001
	DR-DQ	0.031	<0.001	<0.001
	DPB1	0.781	0.215	0.355
Hispanics	DQA1	0.074	---	---
	DQB1	0.41	0.003	0.16
	DRB1	0.63	<0.001	0.16
	DR-DQ	0.29	<0.001	0.02
	DPB1	0.024	0.403	0.026
African-Americans	DQA1	---	0.89	---
	DQB1	---	0.34	---
	DRB1	---	0.001	---
	DR-DQ	---	<0.001	---
	DPB1	---	0.38	---

^aSignificant test results are in bold

Table 5. HLA alleles and haplotypes associated with severity of coccidioidomycosis, by ethnicity

Locus	Caucasian			Hispanic			African-American
	S v. M ^a	S v. C	M v. C	S v. M	S v. C	M v. C	S v. C
a. Alleles associated with decreased risk							
DQB1	*0402 ^b *0504 ^b	*0604 ^b	*0604 ^b		*0302 ^b		
DRB1			*0301 ^c *1104 ^b		*0407 ^c *0408 ^b		*1501 ^c
DRB1-DQB1	*0700- *0303^b	*0301-*0201^b *0404- *0302^b *0700- *0303^b *1302- *0604^b	*0301-*0201^b *1104- *0301^b		*0407- *0302^c *0700- *0201^b *1101- *0301^c *0101- *0501^b	*0301-*0201^c *1402- *0301^c	*1501- *0602^c
DPB1						*0501 ^b	
b. Alleles associated with increased risk							
DQB1	*0602 ^b	rare ^b	*0504 ^d rare ^d		*0602 ^c *0603 ^b *1103 ^c *1301 ^c *1406 ^c rare ^b		*1301 ^c *1503 ^c rare ^c
DRB1			*0303 ^c *1301^b rare ^d				
DRB1-DQB1	*1301-*0602^b	*1301-*0602^d *0700- *0201^b rare ^d	*1301-*0504^c *1302- *0201^c *0303- *0201^c rare ^b		*0700- *0301^c *1301-*0603^b *1302- *0301^c *1406- *0301^c rare ^b	rare ^c	*1301-*0501^c *1503- *0602^c
DPB1				*0101 ^b *0201 ^b		rare ^b	

^aS, severe cases; M, mild cases; C, outside controls

^bp < 0.05

^cp < 0.01

^dp < 0.001

Among African-Americans, only DRB1 showed overall allelic heterogeneity ($p = 0.001$). DRB1*1301, *1501, and *1503 each contributed to the risk for severe disseminated disease (OR = 4.2, $p = 0.008$; OR = 0.1, $p = 0.002$; and OR = 10.2, $p = 0.01$, respectively). Comparing haplotype frequencies increased the significance of these differences ($p < 0.001$). DRB1-DQA1-DQB1 haplotypes *1301-*0102-*0501 and *1503-*0102-*0602 were associated with higher risk (OR = 10.4, $p = 0.003$ and OR = 10.2, $p = 0.01$, respectively), and *1501-*0102-*0602 was associated with lower risk (OR = 0.1, $p = 0.003$) of severe disseminated disease. Rare alleles of DRB1 and DRB1-DQB1 haplotypes were consistently associated with increased risk in all three ethnic groups (Table 5).

The joint effects of HLA DRB1*1301 and blood group A or B phenotype on progression to disseminated disease were tested in Hispanics, which had substantial ABO effects. When severe cases were compared with mild cases, an effect persisted for the A phenotype (OR_{MH} = 0.30, $p = 0.02$, data not shown).

Discussion

Except for the Native American genetic component of the Hispanic (Mexican-American) population, the ethnic groups examined show no evidence of an evolutionary response to the pathogenic threat of *C. immitis*. Despite this fact, evidence is presented that for each ethnic group, allele and haplotype distributions at HLA and ABO genes vary according to disease outcome. Substantial differences at these loci were identified in the risk for symptomatic disease after infection with *C. immitis*. The differences in susceptibility associated with ethnic background can be attributed to genetic influences. In certain instances, identical HLA alleles were observed among Hispanic, Caucasian, and African-American persons. In contrast, only Hispanic participants demonstrated ABO effects.

Effects could be observed if controls inadequately represent cases. Controls were matched as closely as possible to cases by ethnicity and geographic proximity. Caucasians were defined as persons of "mixed European" descent, while Hispanics were defined as "Mexican-Americans." The African-Americans were persons with "European-African admixture." For ABO comparisons, all case patients were from California. For HLA,

all Hispanic and Caucasian persons were from California.

The lack of cases of mild disease noted among African-Americans may be due to ascertainment bias or biologic cause. Severe cases, which require medical management, are much more likely to come to medical attention and be identified by the study. Because mild infections are self-limiting, some persons may not seek medical care and may not be identified. In a population-based surveillance study of CM in Kern County between 1995 and 1996 (11), African-Americans comprised 6% of all mild cases and 22% of all disseminated cases (Table 1). By comparison, Hispanics comprised 38% of mild cases and 39% of disseminated cases in the surveillance study versus 39% and 46%, respectively, in the present study. Thus mild cases among African-Americans are underrepresented in this study by approximately fourfold.

Patients with mild disease may respond differently, depending on ethnic background, in seeking treatment, use of medical care, or response rate to study participation. Statistics on use of health-care services in the United States during 1996-1997 showed that the time since last physician contact was nearly identical for Caucasians and African-Americans. Overall, African-Americans had similar numbers of ambulatory health-care visits per person per year (3.3 for Caucasians versus 3.9 for African-Americans) (29). However, a greater proportion of visits by African-American patients was to emergency rooms or hospitals as outpatients (an average of one visit per person per year for African-Americans versus 0.6 for Caucasians) (29). If this pattern of health-care use is similar in Kern County, then the >10:1 difference in the frequency of mild cases among Caucasians versus those among African-Americans in this study cannot be explained by ascertainment bias.

An alternative explanation is that mild CM is intrinsically rare among African-Americans compared with Hispanics and that most exposures to *C. immitis* in African-Americans result in severe disseminated disease. The surveillance study indicates that mild cases are more rare among African-Americans.

ABO Effects

ABO blood group is one of the first human genetic polymorphisms identified (30). Numerous

studies (17,31) have examined the relationship between ABO and disease, with inconclusive results. The distribution of A and B antigens in the body, the differences between the two alleles (32), and the existence of A, B, and O alleles in aboriginal human populations worldwide (except the New World) point to the evolutionarily demonstrated functional importance of this system. The association of CM with blood group phenotypes between the mild and severe cases among Hispanics, as well as between these cases and controls, again suggests a direct role for blood group antigens in disease susceptibility.

Previous studies reported that the B blood group was more frequent among persons with severe disseminated cases than among those with mild cases (7,16). These analyses did not consider ethnicity. Our results among Hispanics support earlier findings. However, the frequency of the B phenotype among severe cases (14%) and mild cases (0%) is lower than the frequency (31%) reported in the Hispanic population from San Francisco (17). Assuming the controls were adequately matched to cases, the lack of blood group B in mild cases suggests perceived risk when compared with severe cases. For Caucasians, an opposite (nonsignificant) trend is observed: the frequency of blood group B phenotypes in the population is approximately 14%, the same as among persons with mild cases in this study, and relatively fewer persons with severe cases (8%) have this antigen. The frequency of B phenotypes among African-Americans with severe cases (20%) is similar to that observed in the control population (23.4%) (17).

The A blood group phenotypes are more frequent among Hispanics with cases than among controls, but less frequent among those with severe cases than mild cases. This antigen appears to have the opposite effect on disease expression and severity to that of the B antigen.

Culture filtrates of *C. immitis* were reported to contain blood group A activity (33). If this is true, *C. immitis* A-like polysaccharides may be adsorbed to the surface of host cells and cross-react with A antibodies to give false positivity. Certain instances of acquired B expression have been documented in persons with advanced age or disease (30). Also noted is cross-reactivity of anti-B antibodies with polysaccharides from *Escherichia coli* O₈₆, which can induce false B reactivity in infected persons (30). Hardy-Weinberg genotypic frequencies for the

ABO system were in equilibrium among Hispanics, an observation that does not support these explanations.

Alternatively, the immune systems of persons with A phenotypes may not recognize the A-like coccidioidal antigens as foreign. This may allow infection to be established without immunologic challenge to these antigens, leading to increased risk for symptomatic disease. The lower frequencies of B phenotypes may reflect the increased frequency of A phenotypes in this study.

HLA Effects

When analyzing HLA allele and haplotype distributions across ethnic groups, one looks for unifying themes that might implicate a particular allele or haplotype in a universal role for susceptibility in all groups. Such a finding would suggest that the set of molecules themselves is functionally responsible for the observed associations. The specificity of class II molecules depends on the alleles of the heterodimer subunits, which differ in their combined ability to recognize antigenic peptides. HLA polymorphisms defining allelic variation generally occur in the amino acid sites found in or near the peptide binding groove, thus affecting the interaction of the HLA molecule with the peptide or with the T-cell receptor. Some HLA alleles bind and present particular antigens to T cells better than other alleles. In this context, persons who present coccidioidal antigens more effectively to T cells may be able to eliminate the fungus before the disease progresses to more severe forms.

We found that the DRB1*1301 allele was associated with increased risk for disease in comparisons of mild or severe cases versus controls among all three ethnic groups. However, the DQA1-DQB1 portions of the haplotypes associated with this allele and with risk for each ethnic group were not the same. This suggests that the DRB1*1301 allele itself might be responsible for increased risk for symptomatic disease, regardless of ethnic background. DRB1*1301 has previously been associated with reduced risk for perinatal HIV infection (34,35) and decreased risk for progression of perinatally acquired HIV disease (36).

For both Caucasians and African-Americans, the strength of the DRB1*1301 association increases when more specifically defined by the

DRB1-DQA1-DQB1 extended haplotype. For African-Americans with severe cases, this haplotype is *1301-*0102-*0501 (OR = 10.4, $p = 0.003$ vs. controls). Among Caucasians with severe cases, the extended haplotype is *1301-*0103-*0602 (OR = 35.0, $p = 0.0003$ vs. controls) and for mild cases is *1301-*0103-*0504 (OR = 18.1, $p = 0.002$ vs. controls). DQA1*0103 alone is associated with increased risk for disease. However, the haplotype *1301-*0103-*0603 is common among both cases and controls and neutral for CM risk. Among Hispanics with severe cases, the marker for severe disease risk is the extended haplotype *1301-*0103-*0603 (OR = 3.6, $p = 0.05$ vs. controls). These data suggest that DRB1*1301-DQA1*0103 may predispose Caucasians to symptomatic infection, but this risk is modified by DQB1 alleles. DQB1*0603 may negate this risk in Caucasians, while *0504 decreases or *0602 increases the risk for progression to severe disseminated disease. This stepwise mechanism is not evident from results for the other ethnic groups.

For Caucasians and Hispanics with cases, DRB1*0301-DQB1*0201 was associated with lower risk for disease than among controls, consistent with a protective effect of this haplotype. The DRB1*0301 allele has been associated with autoimmune disease risk in Caucasians (37). Among African-Americans, the *1501-*0602 haplotype was associated with lower risk for severe disease.

Another common theme observed in these data is that differences can be attributed in several comparisons to rare alleles or haplotypes present in a higher proportion of cases (either mild or severe) than controls. Since they otherwise share no particular molecular features, this may represent absence of alleles that specifically protect from infection by *C. immitis*.

Lack of consistency for HLA associations across ethnic groups does not preclude involvement of these alleles in specific populations, as HLA effects may produce disease susceptibility across ethnic groups as well as within one ethnic group. For example, HLA class II haplotypes predispose to insulin-dependent diabetes mellitus among Caucasian and Japanese (38). Among Caucasians, DR3 haplotypes are highly significantly associated with disease. This is not true among the Japanese, because DR3 haplotypes are extremely rare in the Japanese population, in which the associated haplotypes are DR4 and

DR9. Thus, HLA alleles and haplotypes may be associated with disease in only one ethnic group.

Future studies could better address the risk for symptomatic CM by recruiting controls who are resistant to disease, i.e., persons with positive coccidioidal antigen skin tests who were not hospitalized for their infections. This selection would identify a more suitable control group for comparison with patients who have either mild or severe forms of CM in investigating risk factors for disease progression.

Our data support the hypothesis that host genes, in particular HLA class II and ABO blood group, play a complex role in susceptibility to severity of CM. Identifying genes that influence risk for developing severe disseminated CM may aid prevention efforts by targeting persons at high risk for disseminated disease who should be monitored closely after potential exposures, and may also aid in study design to test potential vaccines for efficacy, once candidate vaccines become available (39-41).

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