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Human Leukocyte Antigen (HLA)-B, DRB1, and DQB1 Allotypes Associated with Disease and Protection of Trachoma Endemic Villagers

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Abstract

Purpose—Trachoma remains the leading preventable infectious cause of blindness in developing countries. Human leukocyte antigen (HLA) associations with ocular disease severity and persistent *Chlamydia trachomatis* infection of Tanzanians living in trachoma-endemic villages were examined to determine possible protective candidate allotypes for vaccine development.

Methods—Buccal swab scrapes were taken from subjects in the Trichiasis Study Group (TSG), which studied females only, and the Family Trachoma Study (FTS), which compared persistently infected probands who had severe disease with disease-free siblings and parents. DNA was purified for polymerase chain reaction sequence-specific oligonucleotide identification of HLA-DRB1, DQB1, and B allotypes. Infection was detected from conjunctival scrapes using a *C. trachomatis*-specific PCR-enzyme immunoassay for the *MOMP-1* gene.

Results—In the TSG, DR*B11 (odds ratio [OR], 0.48; 95% confidence interval [CI], 0.26 – 0.90; $P = 0.02$) was significantly associated with lack of trichiasis, whereas HLA-B*07 (OR, 3.26; 95% CI, 1.42–7.49; $P = 0.004$) and HLA-B*08 (OR, 5.12; 95% CI, 1.74 – 15.05; $P = 0.001$) were associated with trichiasis. In addition, HLA-B*14 was significantly associated with inflammatory trachoma + follicular trachoma (OR, 3.76; 95% CI, 1.70 – 8.33; $P = 0.04$). There were no significant allele frequencies for the FTS.

Conclusions—The data suggest that HLA-DRB*11 may offer protection from trichiasis in trachoma hyperendemic villages. Complete allotype identification and designation of its respective protective $CD4^+$ T-cell antigens could provide a testable candidate vaccine for blindness prevention.

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Additionally, buccal swab DNA was sufficiently stable when acquired under harsh field conditions and stored long term in the freezer for low-resolution HLA typing.

The intracellular bacterium *Chlamydia trachomatis* causes trachoma, which is found in 55 endemic countries encompassing 10% of the world's population. Approximately 84 million persons have active infection; 8 million are blind from it, and an estimated 7.6 million have trichiasis, the sequel to blindness.[1] Those affected include some of the world's most impoverished and vulnerable people, for whom blindness is also associated with increased risk for death.[2-4] In addition, the annual estimated loss in productivity is approximately 2.9 billion US dollars.[1] Intervention measures of the SAFE program (surgery, antibiotics, facial cleanliness, and environmental change) have made some gains in prevention of infection, disease, and blindness.[5] However, it is clear that an efficacious, economical, and sustainable intervention, such as a vaccine, is desirable.[6,7]

Most Tanzanian village children have experienced trachoma before they are one year of age, and some represent a pool of active disease.[8,9] In older Tanzanian children and adults who share similar epidemiologic characteristics, persistent infection, severe inflammation or scarring, and trichiasis have been identified, whereas others have minimal or no disease and infection in them is cleared rapidly.[10-13] These field studies suggest genetic resistance to chlamydia reinfection and severe disease in trachoma.

In animal studies,[14-19] HLA class I- and II-restricted CD-effector cells recognize chlamydia antigens in vitro, and some facilitate *C. trachomatis* clearance with demonstrated immune memory. Therefore, identifying protective candidate HLA allotypes in human studies of trachoma can serve as a platform for testing computational models of predictive peptide binding for validation.

In the present study, we have taken advantage of the earlier observation by our group of differential response to chlamydia infection in terms of clearance and disease. A candidate protective HLA allele, DRB1*11, against trichiasis has been identified using buccal swab DNA as source material.

Methods

Subjects

Two subject groups were included in this study. The first was the Trichiasis Study Group (TSG), whose subjects were culled from a longitudinal study started in 1989 on the development of scarring and trichiasis in women ($n = 4932$).[20] In 1996, a subset of 186 infected and 186 uninfected women were randomly chosen from a population-based sample of women and girls aged 16 and older from 11 villages in Kongwa district, Dodoma region, Tanzania.[11] At follow-up in 1999, 74 subjects were infected and 85 were uninfected; 73% of the subjects were infected in both 1996 and 1999 with the same chlamydia *ompA* genovar. Those who had chronic infection were more likely to have had trichiasis, scarring, and active trachoma in 1996.[11,12] Overall, there were 127 samples, and DNA was suitable for analysis in 122. Cases were women and girls with trichiasis ± inflammatory trachoma (TI) ($n = 21$), and controls were women and girls with no or minimal disease (nonscarring, noninflammatory follicular trachoma, with or without infection [$n = 77$]).

In the second group, the Family Trachoma Study (FTS), 15 families from one village were chosen because they had persistently infected children (probands) at three time points in a 1-year period ($n = 15$). *OmpA* genotyping data indicated that this was likely persistent infection or reinfection with the same strain,[21] suggesting an inability to mount a protective response. These children constituted the cases. The controls were meant to be their parents ($n = 12$) and

siblings (n = 40) who had minimal or no disease (ie, two parents had follicles, but no control had inflammation, scarring, or trichiasis). The intention was to conduct sibling pair analysis for the phenotype of severe trachoma/protection and HLA type.

Disease was classified using the simplified World Health Organization clinical grading scheme of the tarsal conjunctiva as follows: no trachoma (TN), ≥5 follicles of at least 0.5 mm each as follicular trachoma (TF), ≥50% of normal deep tarsal vessels obscured because of inflammatory thickening of upper tarsal conjunctiva as inflammatory trachoma (TI±TF), and at least 1 eyelash touching the cornea or evidence of recent removal of in-turned lashes as trichiasis (TT).[22] Informed consent was obtained for immunogenetic studies in 1996 in accordance with the Declaration of Helsinki and was approved by the institutional review board at Johns Hopkins University.

Chlamydial DNA

A previously published and validated polymerase chain reaction-enzyme immunoassay for a conserved region of the MOMP-1 gene was used to detect *C. trachomatis* infection from lysed tarsal conjunctival scrapes.[23] This study also describes the collection and processing of ocular samples.

Every fifth PCR sample consisted of a swab inserted into PCR buffer by the sampler in the field. The contamination rate was 0.1%.

Buccal DNA for HLA Studies

Negative controls were sample buffer with an uninoculated brush added by the sampler in the field; the contamination rate was 0.05%. Briefly, cytology brushes containing DNA were placed 0.6 mL of 50 mM NaOH and 0.2 mM EDTA. Samples were then incubated at 80°C for 10 minutes, returned to room temperature, and neutralized with 50 μL of 1 M Tris-Cl. For longer storage at −80°C, equal volumes of 90% vol/vol glycerol/water was added to the lysate.[24-26] All the samples were further purified using a commercially available DNA column chromatography-assisted strategy (QIAamp DNA Blood Mini Kit; Qiagen, Valencia, CA).

Amplifications of the *HLA-DRB1*, *DQB1*, and *B* genes were performed with 5′-biotin-labeled primers using a commercially available DNA amplification system (GeneAmp PCR System 9700; Applied Bio-systems, Foster City, CA). This was followed by oligonucleotide hybridization and labeling with the fluorescent reporter molecule streptavidin R-phycoerythrin. Hybrids were then analyzed by flow cytometry (Luminex 100; Luminex Corp. Austin, TX), and HLA-typing software (One Lambda, Canoga Park, CA) assisted in the low-resolution HLA genotype determination. HLA data will be submitted to the dbMHC immunology working group (www.ncbi.nlm.gov/gv/mhc/ihwg).

Statistical Analysis

The HLA frequencies of DR, DQ, and B alleles in cases and controls were compared using two-sided Fisher exact test with 2 × 2 contingency tables or, where appropriate, by the χ^2 test with Yates correction. $P < 0.05$ was considered significant, and the Bonferroni correction for multiple tests was applied. Odds ratios (OR) within the 95% confidence interval (CI) was calculated.

Results

Laboratory Methods

Although peripheral mononuclear cells would have been the ideal source material for HLA studies, we were unable to obtain blood from the study participants at the time of this study.

Therefore, buccal swab scrapes provided a noninvasive way to obtain the specimens. Samples had been stored at room temperature in the field for approximately 2 months and were frozen at −80°C for 4 years before processing. The mean purified DNA yield was 23.7 ng/μL (range, 8.3–57.9 ng/μL), and there were no significant differences of yield based on sex, age, or clinical disease (data not shown). Ninety-nine percent of the samples were adequate for low-resolution HLA typing by polymerase chain reaction sequence-specific oligonucleotide. However, half the samples were inadequate for high-resolution allelic determination because of the inability to generate the required larger amplicon.

HLA Associations

The intention of the family study was to perform sibling pair analysis. There were no statistically significant HLA allotype associations for the comparison of index children with their control sibs. Only 11 of 15 families had both a case[11] and sibling controls[26] with DNA of adequate quality. In addition, a father was not identified in 4 of 11 of the families. The paternal DRB1* type was not found in any of the siblings from 3 of 7 remaining families.

The TSG group was next examined, and the clinical and infection prevalence are shown in Table 1. Infection prevalences in each category of TI±TF, TF, no signs of disease (NS), and TT±TI were 24.1%, 8.3%, 12.1%, and 23.8%, respectively. HLA frequencies for TSG in Table 2 indicate that DRB*11 is associated with lack of trichiasis, whereas HLA-B*7 and HLA-B*8 are associated with trichiasis when using case as TT±TI and control as TF and NS. When using TI±TF as a case with TF and NS as a control, HLA-B*14 frequency was significant (OR, 3.76; 95% CI [1.70 – 8.33]; $P = 0.004$), with no “protective” allele found. Four samples from NS and one from TI ± TF could not be typed because of sample inadequacy and were excluded from the frequency analysis. None of the five persons was infected.

Discussion

We have identified HLA-DRB1*11 as a candidate protective allele against trichiasis using DNA from buccal scrape samples obtained under harsh field conditions. Although this methodology has been used by others, to our knowledge it has not been adopted for trachoma field studies under the adverse field conditions found in Tanzanian villages.

This methodology was perfectly suitable for low-resolution HLA typing. However, we were unable to identify the entire HLA allele, likely because of the small amount of starting material in combination with some degree of sample degradation in the field. This resulted in our inability to generate enough DNA for the larger amplicons of HLA-B and -DQ for high-resolution allelic genotyping (4-digit alleles). The repeated attempts to use sequence-specific primer (SSP) and sequence-based typing (SBT) were not successful in amplifying the pertinent exons required for high-resolution genotyping of HLA-DRB*11. Although the reported statistical association in this study was based on low- to medium-resolution HLA typing, it could point to a trend for a legitimate genetic association. The evolution of typing methodologies from serologic to molecular methods confirms this trend. Indeed, many HLA-associated diseases were first reported at the low-resolution level and then confirmed at the allelic level.

We were unable to identify statistically significant HLA alleles that distinguished persistently infected children with severe disease from their control siblings who shared the same environmental exposure, perhaps in part because of the small sample size. More important, the data suggest that some of the sibling relationships might have been half-siblings or possibly non-siblings, which would invalidate using an analysis of sibling pairs discordant for the severe trachoma phenotype. The practice of polygamy and intervillage migration by the adults is prominent in this area of Tanzania. Affected sibling pair analysis has been used to determine

the contribution of HLA class I and class II loci to the development of cervical cancer and human papillomavirus infection.[27] Sibling pair analysis could be useful in HLA and linkage studies for other immune response genes in trachoma with carefully characterized families and with suitable sample size. Other considerations were that some participants in this study lived in villages that had been part of the initial azithromycin treatment trial, but this did not appear to influence the results.[28]

Acquisition of blood samples from the study population would allow us to go forward with identification of the entire candidate allele and to proceed with isolation of restricted $CD4^+$ T cells. It is essential to perform high-resolution typing of HLA-DRB1*11 to validate the association of this allele with the lack of trichiasis. Preliminary data suggest that $CD4^+$ T cells may protect against repeated bouts of infection leading to trichiasis. Additionally, we would be able to further our understanding of the role of linkage disequilibrium (LD) between particular DR-DQ alleles and innate immune markers in LD with HLA-B in ongoing studies. To our knowledge, there have been no studies aimed at identifying anti–*C. trachomatis* $CD4^+$ T cells that are restricted by HLA-DRB*11 in trachoma. The few studies that have been conducted in trachoma have focused on HLA class I–restricted $CD8^+$ effector cells for the outer membrane protein.[14,16,18] Interestingly, one study demonstrated global inhibition of $CD8^+$ T-cell activation in mice during primary and secondary chlamydia infection.[29] In our study, we found that HLA-B*07 and HLA-B*08 were associated with trichiasis and that HLA-B*14 was associated with inflammatory follicular disease. As mentioned, it would be necessary to identify the complete allele before attempting to elucidate the contribution of the associated class I–restricted $CD8^+$ cells to chlamydia pathogenesis.

The association of DRB1*11 with protection has not been found in other chlamydia infection association studies. Recurrent chlamydia genital and tubal factor infertility and blinding trachoma were associated with a variety of class II alleles.[30,31] DRB1*11 was found to be associated with protective effects in other infections involving intracellular pathogens. For example, DRB1*11-DQB1*03 in LD was found more often in controls than in those with tuberculosis (TB), suggesting a link to TB resistance.[32] DRB*11 has figured in an association with protection in the mild, restricted form of paracoccidiomycosis and mild liver damage in hepatitis C infection.[33,34] Persons carrying HLA-DR11 express HLA-DR 52 and are in LD with HLA-DQ7 serotypes. The DQ7 serotype corresponds to the allotype DQB1*03, which includes more than 20 alleles. When DR11 is analyzed as part of its linked DQB1*03 allotype, the DRB1*1102-DQA1*0505-DQB1*0301 haplotype is associated with hepatitis B virus persistence.[35]

One of our goals was to compare HLA frequencies in several trachoma endemic regions with those obtained in the present Tanzanian study. There is a significant difference between cases/10,000 endemic population in West (1002) and East Africa (1031) compared with North (514) and South Africa (217) (WHO Global Health Atlas). This is because of the huge burden of severe disease in Ethiopia, Tanzania, and Burkina Faso. Tanzania is considered a mesoendemic region, with the TI/TF prevalence 5% to 40% in those younger than 6 years of age in 67 villages of 2500 to 10,000 inhabitants (according to the institutional review board of the Tanzania National Institute for Medical Research; Bobo L, personal communication, 2008). Gambia is considered hypoendemic because of a prevalence TI/TF of less than 10% in clusters of those younger than 10 years in 114 areas of 600 to 800 inhabitants (according to the institutional review board of the Joint Gambia Governor's Medical Research Council Ethics; Bobo L, personal communication, 2008). On the other hand, Ethiopia is described as hyperendemic with a TI/TF greater than 40% in those younger than 10 years from 200 villages of 400 inhabitants, and trichiasis occurs at an earlier age (according to the institutional review board of the University of Gondor, Ethiopian Science and Technology; Bobo L, personal

communication, 2008). It is unclear how the endemicity differences in these countries would contribute to the evolution of protective or pathogenic HLA alleles.

It is believed that evolving pathogens mainly evade presentation by the most common major histocompatibility complex (MHC) alleles in the host population by providing selective pressure for a large variety of rare MHC alleles.[36-38] However, the more frequent allele in the present study, DRB1*11, was associated with lack of trichiasis and was significantly more represented in Tanzania (32.1%) than in the rest of East Africa (17.5%), West Africa (12%), and North Africa (17.2%) (dbMHC MHC database and Immunology/Histocompatibility Working group; www.ncbi.nlm.nih.gov/gv/mhc/ihwg.cgi). Unfortunately, at present there are sparse data on HLA frequencies or linkage disequilibrium for infectious disease associations for African countries. This makes evaluation of the dynamics of chlamydia-MHC polymorphism in trachoma and how it would affect vaccine efficacy difficult across African countries.

In *C. trachomatis*, recombination occurs within the immunodominant *ompA* gene; there is little information on the mutability of other immunoaccessible chlamydia proteins.[39,40] Pathogen-MHC coevolution is a dynamic process, but it is unknown whether HLA results from one geographic region can be generalized to another and how this process would affect vaccine efficacy. Additionally, it is unclear what the effects of coinfection with other pathogens would have on this process.

Another drawback of our study was that we did not identify who was coinfected with important pathogens, such as HIV-1, HIV-2, *Mycobacterium tuberculosis*, or *Plasmodium* species. In addition, the study participants were not assessed for malnutrition, lymphopenia, or vitamin A status, factors that are important in immune and ocular health. Therefore, it is possible that these variables might have biased the results for weaker HLA associations.

Recently, the coexistence of *Chlamydiaceae* species in trachoma-endemic regions in Nepal has been identified, as has their association with clinical severity and their involvement in the immune response to Hsp60.[41] Similarly, previous exposure to *Chlamydia pneumoniae*, a common respiratory pathogen, was hypothesized to prime a Th1 T-cell response to certain *C. trachomatis* antigens in the context of HLA-DRB1*0401.[42] If this is true in other trachoma-endemic populations, such as in our Tanzanian study population, it will be important to determine which species should be taken into consideration for vaccine development using HLA-DRB1*11.

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Table 1

Clinical and Infection Prevalence of the Trichiasis Study Group

	Category				
Infection	**TI ± TF**	**TF**	**NS**	**TT ± TI**	**Total**
Chlamydia	7	1	7	5	20
No *Chlamydia*	22	11	58	16	107
Total	29	12	65	21	127

TI ± TF, inflammation with or without follicles; TF, follicles with no scars or inflammation; NS, no signs of disease; TT ± TI, trichiasis with or without inflammation.

Table 2

Allele Frequencies for the Trichiasis Study Group

HLA Gene	Allele	Case (*n*)	Control (*n*)	OR (95% CI)
DRB1		(82)	(369)	
	1	5	29	0.76 (0.33–1.72)
	3	11	40	1.27 (0.70–2.30)
	4	4	8	2.31 (0.83–6.42)
	7	2	16	0.55 (0.16–1.90)
	8	2	8	1.13 (0.30–4.19)
	9	6	11	2.57 (1.09–6.05)
	10	7	18	1.82 (0.85–3.88)
	11	**14**	**110**	**0.48 (0.26–0.90)**[*]
	12	3	11	1.24 (0.41–3.67)
	13	10	49	0.91 (0.50–1.66)
	14	2	1	9.2 (1.23–69.00)
	15	15	65	1.05 (0.62–1.76)
	16	1	3	1.51 (0.22–10.10)
DQ1		(80)	(372)	
	2	13	41	1.52 (0.86–2.67)
	3	14	94	0.61 (0.36–1.02)
	4	4	27	0.66 (0.27–1.62)
	5	18	80	1.03 (0.63–1.70)
	6	31	130	1.13 (0.75–1.70)
HLA B		(76)	(360)	
	7	**10**	**16**	**3.26 (1.42–7.49)**[†]
	8	**7**	**7**	**5.12 (1.74–15.05)**[‡]
	13	1	5	0.93 (0.15–5.65)
	14	4	14	1.35 (0.52–3.50)
	15	11	46	1.13 (0.62–2.04)
	18	5	34	0.66 (0.29–1.49)
	35	5	22	1.06 (0.46–2.45)
	39	3	6	2.39 (0.74–7.74)
	41	1	3	1.56 (0.23–10.44)
	42	3	19	0.73 (0.26–2.06)
	44	6	20	1.43 (0.65–3.15)
	45	4	44	0.40 (0.14–1.15)
	49	2	13	0.71 (0.20–2.50)
	51	1	7	0.66 (0.11–3.84)
	53	4	41	0.43 (0.18–1.04)
	57	1	11	0.42 (0.07–2.35)
	58	6	45	0.59 (0.28–1.24)
	81	2	7	1.34 (0.35–5.06)

[*] $P = 0.02$;

[†] $P = 0.004$;

[‡] $P = 0.001$.

Values shown in bold are considered significant ($P < 0.05$).