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ORIGINAL ARTICLES — Clinical science

HLA antigens in Omanis with blinding trachoma: markers for disease susceptibility and resistance

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Accepted for publication 13 January 1997

Abstract

***Aim*—To determine the presence of HLA antigens in people with blinding trachoma.**

***Methods*—Fifty Omanis with blinding trachoma were serologically typed for HLA A, B, C, DR, and DQ antigens and DNA typed for class II DRβ and DQβ alleles and compared with a population of 100 healthy controls.**

***Results*—χ^2 analysis of serological reactions did not reveal any significant differences in HLA antigen frequencies after correction of probability, although DR4, DR7, and DR53 were completely absent in the patients and all of the patients were HLA DQ1 positive. In the case of DQ1 the relative risk was 22.6 (95% confidence interval of 20.7–24.7). Class II DNA low resolution DRβ typing showed a significant increase in HLA DR16 (p_c = 0.036, relative risk = 3.8) and a significant decrease in HLA DR53 (p_c = 0.018, relative risk = 0.05).**

***Conclusion*—The finding that HLA DR16 (a DR2 subtype) is associated with susceptibility to blinding trachoma, a disease that is caused by an intracellular micro-organism, is consistent with reports of an HLA DR2 association with leprosy and tuberculosis, diseases also caused by an intracellular micro-organism. Similarly, resistance to leprosy is associated with HLA DR53 as is the case with blinding trachoma described here. It is postulated that HLA DR2 or subtypes in association with HLA DQ 1 may enable an intracellular micro-organism to enter the cell or are involved in presentation of peptides derived from intracellular micro-organisms to T lymphocytes initiating a delayed hypersensitivity or autoimmune reaction. These findings are the first report that genetic factors are of major importance in the development and protection against blinding trachoma.**

(*Br J Ophthalmol* 1997;**81**:431–434)

Trachoma is a disease that actively affects at least 500 million individuals worldwide. At least two million of these people are blind from trachoma with many more having a significant visual handicap.[1] It is the second most common cause of blindness after cataract.[2] It is initiated by repeated infections with *Chlamydia trachomatis* and in endemic areas it is an infectious disease of early childhood, promoted by low socioeconomic status with deficient community and personal hygiene. Most children are infected by the age of 1–2 years and from the age of 5 years the number of detectable *C trachomatis* positives declines steadily. A significant number of these children show no inflammatory reaction and the eyes appear normal.[3] In later childhood and in adults, infectious and inflammatory disease becomes less prevalent, and in a large proportion of these no *C trachomatis* can be detected even using the polymerase chain reaction to amplify chlamydial DNA.[4] Even in the later stages of trachoma, which are characterised by trichiasis and corneal opacities, demonstration of *C trachomatis* is the exception. The finding of infection without disease and disease without infection suggests that the immune response to the infection may determine the clinical status.[5] Infections with *C trachomatis* are self limiting but repeated reinfections from the environment give the disease its apparent chronicity. After each infection the organism is cleared and the persistent clinical response may be a delayed hypersensitivity reaction directed against residual *C trachomatis* antigens, resulting in prolonged inflammation. Furthermore, inflammatory trachoma has several factors that are also common to organ specific autoimmunity. The expression of MHC class II antigens by the conjunctival epithelium in inflammatory trachoma has been reported.[6]

The clinical picture of trachoma varies from a very mild condition with hardly any symptoms to a severe and blinding disease.[1] Within a community there may be striking differences between families in the prevalence and severity of disease.[3] The degree of inflammatory immune response appears to be partly host determined, since even in areas where trachoma is holoendemic, only a subset of individuals develop severe scarring of the upper conjunctiva with trichiasis and corneal opacities leading to blinding trachoma.[7] This

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Table 1 Significant associations with and without probability correction of HLA serologically detected antigens in patients with blinding trachoma

HLA antigen	Blinding trachoma (n=50) pos	neg	Controls (n=100) pos	neg	RR	χ^2	p Value	p_c*
HLA-A30	1	49	19	81	0.12	7.33	0.007	0.4235
HLA-A32	16	34	17	83	5.17	4.37	0.037	2.2570
HLA-DR2	46	4	73	27	3.86	6.76	0.009	0.5654
HLA-DR4	0	50	11	89	0.07	5.89	0.015	0.8912
HLA -DR7	0	50	8	92	0.10	4.34	0.035	2.1319
HLA-DR53	0	50	15	85	0.05	7.72	0.006	0.3483
HLA-DQ1	50	0	82	18	22.64	8.97	0.003	0.1933

*p_c=probability × no of comparisons (79).

evidence may indicate that there is a genetic susceptibility in individuals who develop blinding trachoma. Antigens of the major histocompatibility complex (MHC) have been reported to be associated with a broad spectrum of human disease and in particular diseases associated with aberrant immunity. We could find only one other report in the literature that looked at the frequency of human leucocyte antigens (HLA) in trachoma.[8]

This report describes the frequency of HLA antigens in Omanis with blinding trachoma.

Material and methods

Fifty patients with blinding trachoma defined by the presence of trachomatous trichiasis and corneal opacities in accordance with the WHO 1987 trachoma grading system[9] were typed for HLA antigens. All patients were examined and identified by the same consultant.

The frequency of HLA antigens in these patients was compared with 100 Omani controls for detection of HLA class I and II antigens by serology and class II DRβ1 and DQβ1 by DNA analysis. The Omani controls were healthy volunteer blood donors, university students, potential kidney or bone marrow donors. These donors, like the patients with blinding trachoma, came from all parts of Oman, and were considered ethnically representative of the normal population. None of the patients or donors were related.

For serological HLA-ABC and DR typing 15–20 ml of blood taken into EDTA was used. Lymphocytes were separated by density gradient centrifugation and then the class II positive cells (predominantly B lymphocytes) separated using magnetic beads (Dynabeads, Dynal, Skoyen, Norway). The supernatant containing T cells was used for ABC typing and the purified B cells for DR (class II) typing. HLA typing was performed using a modified two stage cytotoxicity technique with ethidium bromide/acridine orange staining and observation with a semiautomated fluorescent microscope.[10]

The following serological specificities could be determined (79 in total):

A locus: 1, 2, 3, 9, 10, 11, 19, 23, 24, 25, 26, 28, 29, 30, 31, 32, 33, 34, 36.

B locus: 5, 7, 8, 12, 13, 14, 15, 16, 17, 18, 21, 22, 27, 35, 37, 38, 40, 41, 42, 44, 45, 48, 49, 50, 51, 52, 53, 55, 57, 60, 61, 62, Bw4, Bw6.

C locus: w1, w2, w3, w4, w5, w6.

DR locus: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, DRw52, DRw53.

DQ locus: 1, 2, 3, 7.

For class II DNA typing, the DNA was extracted from 2 ml of EDTA blood using either a commercial kit (Nucleon, Scotlab, UK) or a local method using a rapid mini salting out technique based on that described by Miller *et al.*[11] The separated DNA was amplified in a thermal cycler, following the manufacturer's instructions, with class II primers defining DRβ1and DQβ1 (Dynal low resolution SSP, Dynal, Skoyen, Norway) together with PCR buffer, taq polymerase, and DNA nucleotides (Gibco BRL, Paisley, UK). The products were run on 1.2% agarose gels containing ethidium bromide, examined under ultraviolet illumination, and the class II specificities identified. The following DR and DQ specificities could be determined (32 in total).

DNA DR: 1, 2 (15, 16), 3 (17, 18), 4, 5 (11,12), 6 (13, 14), 7, 8, 9, 10.

DRw: 51, 52, and 53.

DNA DQ: 1 (5.1, 5.2, 5.3, 6.1, 6.2, 6.3, 6.4, 6.7, 6.9).

2 (2.1, 2.2).

3 (3.1, 3.2, 3.3, 3.4, 3.5).

4 (4.1, 4.2).

Statistical analysis was carried out using the χ^2 test or the Fisher's exact test depending on the numbers involved. The probability was corrected for the number of comparisons made using the Bonferroni correction, obtained by multiplying the number of antigens tested for (79 for serology and 18 for class II DNA DRβ1, and 14 for DNA DQβ1) to correct for any chance associations (p_c).[12] The strength of the association was estimated by calculation of the relative risk (RR) using Haldane's modification for small numbers when appropriate.[13] The 95% confidence intervals (CI) of the relative risk were calculated using the method based on the logarithm of the standard error with reference to a normal distribution.[14]

It should be emphasised that the calculation of relative risk can be misleading when the numbers of positives/negatives in both patients and controls are low.

Results

The class I and class II results determined by serology that reached statistical significance using χ^2 analysis (uncorrected probability) are shown in Table 1 together with the corrected probabilities.

In relation to HLA serology none of the antigens were significantly associated with blinding trachoma using χ^2 analysis, when the probability was corrected. However, calculation of relative risk, when adequate numbers were available, showed that susceptibility was associated with HLA-A32 (RR = 5.17, 95% CI 2.86 to 9.34), DR2 (RR = 3.86, 95% CI 3.35 to 4.45), and DQ1 (RR = 22.6, 95% CI 20.72 to 24.69). Resistance to trachoma was associated with HLA-A30 (RR = 0.12, 95% CI 0.02 to 0.87), DR4 (RR = 0.07, 95% CI 0.009 to 0.53), DR7 (RR = 0.10, 95% CI 0.01 to 0.78), and DR53 (RR = 0.05, 95% CI 0.006 to 0.35).

All the class II results for DNA DRβ1 and DQβ1 are shown in Tables 2 and 3. DNA typing revealed that the DR16 split of DR2 was

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Table 2 Class II DNA HLA DR β1 in trachoma patients and controls

DR antigens	Trachoma patients (n=46) pos	neg	Controls (n=100) pos	neg	RR	χ^2	p Value	p_c
DR1	2	44	2	98	2.21	0.68	0.794	14.292
DR103	0	46	0	100	—	—	—	—
DR2:								
combined	42	4	72	28	3.71	6.86	0.009	0.162
15	6	40	22	78	0.56	1.63	0.202	3.636
16	39	7	58	42	3.82	10.14	0.002	0.036*
DR3:								
17	16	30	27	73	1.44	0.92	0.338	6.084
18	1	45	2	98	1.30	0.005	0.945	17.010
DR4	0	46	13	87	0.07	6.56	0.011	0.198
DR5:								
11	5	41	13	87	0.86	0.13	0.716	12.888
12	0	46	1	99	0.71	0.46	0.469	8.928
DR6:								
13	1	45	8	92	0.36	1.85	0.175	3.150
14	2	44	0	100	11.30	0.65	0.420	7.560
DR7	0	46	7	93	0.13	3.38	0.067	1.206
DR8	0	46	1	99	0.71	0.46	0.496	8.928
DR9	0	46	0	100	—	—	—	—
DR10	2	44	3	97	1.09	0.17	0.678	12.204
DR51	42	4	69	31	4.30	8.60	0.004	0.072
DR52	24	22	46	54	1.27	0.48	0.607	10.926
DR53	0	46	16	84	0.05	10.66	0.001	0.18*

*Statistically significant.

Table 3 Class II DNA/HLA DQ β1 typing of trachoma patients and controls

HLA antigen	Trachoma patients (n=46) pos	neg	Controls (n=100) pos	neg	RR	χ_2	p Value	p_c
DQB*0501	5	41	13	87	0.86	0.132	0.716	10.020
DQB*0502	38	8	63	37	2.67	5.682	0.018	0.252
DQB*0503	2	44	1	99	3.72	*	0.234	3.276
DQB*0601	2	44	8	92	0.61	*	0.506	7.084
DQB*0602	4	42	8	92	1.15	*	0.558	7.812
DQB*0603/0607	3	43	5	95	1.40	*	0.488	6.832
DQB*0604/0609	1	45	2	98	1.30	*	0.682	9.548
DQB*0201/0202	14	32	27	73	1.19	0.184	0.668	9.352
DQB*0301	2	44	11	89	0.44	*	0.159	2.226
DQB*0302	0	46	8	92	0.12	*	0.044	0.616
DQB*0303	1	45	1	99	2.20	*	0.532	7.448
DQB*0304	1	45	0	100	6.62	*	0.315	4.410
DQB*0401	0	46	0	100	2.16	—	—	—
DQB*0402	1	45	3	97	0.92	*	0.625	8.750

*When numbers are small Fisher's exact test applied.

significantly associated with susceptibility to blinding trachoma (p_c = 0.036, RR = 3.82, 95% CI 3.11 to 4.69) and DR53 with resistance to the disease (p_c = 0.018, RR = 0.05, 95% CI 0.01 to 0.36).

In our study, analysis of the frequency of A28 showed an increase in patients (p = 0.037) but this was not significant after appropriate correction (p_c = 2.275).

The HLA association, determined by serological typing, in terms of relative risk, for DR2, DR4, DR7, and DR53 was confirmed by DNA typing.

Discussion

To our knowledge there is only one other report in the literature that has looked at the frequency of human leucocyte antigens (HLA) in trachoma.[8] These authors looked at HLA frequencies in Gambian patients with eyelid scarring. Our patients had more advanced disease in that they had gone on to develop corneal opacification, fitting with the WHO classification for 'blinding trachoma'. The Gambian study found an association of the disease with a subtype of the HLA A28 antigen (HLA A*6802). In our study A28 was not significantly associated with blinding trachoma. We do not know if the patients in our study are comparable with those in the Gambian study and there remains the possibility that the HLA frequencies reported in our study are related specifically to corneal opacification and hence blindness. The other possibility is that the differences may be due to differences in ethnic background.

We have demonstrated a significant association of HLA DR16 (DR2) with susceptibility (RR = 3.82) and DR53 with protection against the disease (RR = 0.05). HLA DR53 is in linkage disequilibrium with both DR4 and DR7, leading to a decrease in both of these antigens although this did not reach statistical significance.

The role of HLA antigens in protection from disease merits further comment. There are two clear examples from the literature. HLA-DR2 and DQ6 (in linkage disequilibrium with DRβ genes encoding DR2) have been reported to protect from type 1 diabetes. This HLA associated protection is dominant with a relative risk of 0.02. Thus, type 1 diabetes rarely develops in DR2/DQ6 individuals.[15]

Protection from severe malaria is reportedly associated with HLA-Bw53.[16] HLA-Bw53 was demonstrated in 15.7% of patients with severe malaria compared with 24.3% in mild malaria controls and 25% in healthy adults. The uncorrected probability was p = 0.04—if corrected by multiplying by the numbers of antigens tested for (45 for class I) the results would have been clearly statistically insignificant (p_c = 1.8) with a relative risk of 0.59. The data presented here clearly show that HLA DR53 is significantly associated with protection from blinding trachoma (p_c = 0.018) and a relative risk of 0.05.

Perhaps more interestingly HLA DR16, a subtype of DR2 defined in this study by DNA, is significantly associated with susceptibility to blinding trachoma. It is worth drawing attention to the fact that two other diseases caused by an intracellular micro-organism—namely, leprosy[17–19] and tuberculosis,[19 20] have been reported to be associated with HLA DR2 or its subtypes. In two of these papers,[18 19] HLA DQ1 was also significantly associated with leprosy and tuberculosis. All the patients in our study were HLA DQ1 positive with a relative risk of 22.6 (95% CI 20.72 to 24.69).

It is conceivable that HLA DR2 (DR15/DR16) and/or DQ1 is a receptor for certain microbial antigens and is a portal for entry of intracellular infections or is responsible for presentation of antigenic peptides derived from intracellular pathogens to the T cell receptor. HLA class II expression has been demonstrated in conjunctival biopsies in patients with active trachoma.[6 21] Thus, these epithelial cells are potentially able to present chlamydial antigens to T cells and potentiate what may become an autoimmune disease or a delayed hypersensitivity reaction.

Furthermore, in Japanese patients with leprosy[17] HLA DR53 was significantly decreased in the patients, supporting our own finding that HLA DR53 is absent in patients

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and may significantly protect from blinding trachoma.

It is possible that HLA DR16 and DQ1, in the absence of DR53, may be part of an antigen presenting complex resulting in blinding trachoma.

To quote from Dawson *et al*[3] 'Within a community there may be striking differences, between families, in the prevalence and severity of the disease. These variations appear to be intimately related to environmental and behavioural factors'. We can now add 'genetic factors' to this quotation and help to explain some of the enigmas of this disease.

Since this manuscript was submitted a report on HLA in patients with trachomatous scarring of the eyelid, but not blinding trachoma, has been published.[8]

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Br J Ophthalmol 1997 81: 431-434
doi: 10.1136/bjo.81.6.431

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