HLA typing in congenital toxoplasmosis

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Abstract

HLA-A, HLA-B, HLA-C, and HLA-D typing was performed in 47 mothers of patients suffering from ocular toxoplasmosis to investigate whether an immunogenetic predisposition exists for developing congenital toxoplasmosis in their offspring. No significant association between any HLA antigen was observed in the mothers of patients with ocular toxoplasmosis, although a total absence of the HLA-B51 antigen was found in this group. HLA-A, HLA-B, and HLA-C typing was also performed in their children (52 patients with ocular toxoplasmosis), to investigate a possible relation between the severity of ocular toxoplasmosis and an eventual immunogenetic factor. In the patients with ocular toxoplasmosis an increased frequency of the HLA-Bw62 antigen was observed in correlation with severe ocular involvement.

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Congenital toxoplasmosis in humans is a serious, sometimes fatal, disease which is frequently associated with ocular abnormalities and psychomotor retardation. Ocular toxoplasmosis is an important cause of blindness and visual handicap in young people. Ocular toxoplasmosis is a leading cause of posterior uveitis and is considered a result of congenital infection although several cases of acquired ocular infection have been reported.¹⁻⁴

Human infection with the parasite *Toxoplasma gondii* occurs by either the congenital or the acquired route. The acquired disease is caused usually after the ingestion of oocysts or tissue cysts and almost all cases are asymptomatic.⁵ However, acquired toxoplasmosis contracted during pregnancy may lead to congenital infection of the fetus. Once maternal immunity has developed, it is believed that all future pregnancies in immunocompetent women are protected from contracting congenital toxoplasmosis.

Congenital infection occurs when the pregnant woman is infected and during the parasitaemia *Toxoplasma* organisms cross the placenta and invade the tissue of the developing fetus.⁶ The frequency of transplacental parasite passage is associated with the moment during pregnancy in which infection takes place.^{6,7} The most important factor, enhancing the transmission of parasites to the fetus, is the infection of the placenta. Except at the gestation time, the duration of the mothers' parasitaemia and its severity influence the placental infection rate and thereby the transmission of

parasites to the fetus. Overall, 40% of children born to mothers with seroconversion during pregnancy become infected.⁸ Other factors influencing the infection rate and clinical presentation should also be taken into account since 60% of infected children are asymptomatic at birth, but frequently develop ocular symptoms during their first 20 years of life.⁹ It is not known why some infected children develop chorioretinitis while others do not.

The association of HLA antigens and severity of infectious disease was reported in malaria.¹⁰ To investigate whether immunogenetic factors are involved in the development of *Toxoplasma* chorioretinitis, HLA studies have been performed but did not reveal any significant associations.¹¹⁻¹³ Until now, these studies were done in congenitally infected patients whereas their infected mothers were not assessed.

The aim of this study was to investigate whether an immunogenetic predisposition exists in mothers for developing congenital toxoplasmosis in their offspring, and also to find out if a relation exists between the severity of congenital toxoplasmosis and this eventual immunogenetic factor.

Materials and methods

We performed HLA-A, HLA-B, HLA-C, and HLA-D locus typing in 47 mothers of patients with ocular toxoplasmosis and HLA-A, HLA-B, and HLA-C typing in 52 patients with ocular toxoplasmosis (children of the above mentioned mothers). In five mothers HLA typing could not be performed because of technical reasons: four blood samples could not be tested because of sample handling mistakes, and one mother withdrew from the study; thus 47 mother-patient pairs were analysed.

The 52 patients with ocular toxoplasmosis included two groups: (1) 42 consecutive patients with *Toxoplasma* chorioretinitis consulting the ophthalmology department of the University Hospital in Amsterdam, and (2) 10 patients with severe congenital toxoplasmosis associated in all cases with ocular and neurological symptoms and psychomotor retardation requiring institutional care. The mother of these patients were approached by letter describing the aims of the study and its background and included a request for a blood sample. The cooperation was obtained in all but one case. The mothers were not ophthalmologically examined. Their children had congenital toxoplasmosis, and therefore the mothers were bound to have contracted acquired toxoplasmosis during their pregnancy.

Table 1 HLA antigen frequencies in 47 mothers of patients with ocular toxoplasmosis

Antigen	Antigen frequency		χ^2	Relative risk	Exact p value
	Mothers (%) (n=47)	Controls (%) (n=2495)			
A-1	21.56	31.08	1.6	0.6	0.206
A-2	50.98	52.72	0.0	0.9	1.000
A-3	31.37	27.70	0.1	1.1	0.752
A-9	7.84	20.41	4.1	0.3	0.043
A-10	7.84	7.01	0.0	1.1	1.000
A-11	15.68	10.30	1.0	1.6	0.317
AW19	35.29	22.24	4.1	1.9	0.043
A-23	0.00	2.73	0.5	0.0	0.479
A-24	7.84	17.78	2.7	0.3	0.100
A-25	1.96	2.04	0.2	0.9	0.655
A-26	3.92	3.89	0.1	1.0	0.752
A-28	5.88	8.99	0.2	0.6	0.655
A-29	7.84	5.22	0.2	1.5	0.655
A-30	6.25	2.89	0.8	2.2	0.371
A-31	6.25	4.30	0.0	1.4	1.000
A-32	7.84	5.74	0.1	1.3	0.752
AW33	1.96	2.01	0.2	0.9	0.655
B-5	1.96	10.98	3.3	0.1	0.069
B-7	17.64	27.33	1.9	0.5	0.168
B-8	31.37	23.56	1.2	1.4	0.273
B-12	15.68	23.33	1.2	0.6	0.273
B-13	7.84	4.68	0.5	1.7	0.479
B-14	0.00	4.68	1.5	0.0	0.221
B-15	25.49	16.63	2.2	1.7	0.138
B-16	5.88	6.65	0.0	0.8	1.000
B-17	9.80	8.13	0.0	1.2	1.000
B-18	7.84	6.86	0.0	1.1	1.000
B-21	3.92	3.28	0.0	1.2	1.000
BW22	7.84	4.93	0.3	1.6	0.584
B-27	5.88	9.26	0.3	0.6	0.584
B-35	25.49	16.24	2.4	1.7	0.121
B-37	1.96	3.66	0.0	0.5	1.000
B-38	3.92	2.65	0.0	1.4	1.000
B-39	1.96	3.82	0.1	0.5	0.752
B-40	11.76	15.58	0.3	0.7	0.584
BW41	3.92	1.40	0.7	2.8	0.403
B-44	17.64	22.51	0.4	0.7	0.527
B-45	0.00	0.84	0.0	0.0	1.000
BW47	0.00	0.48	0.2	0.0	0.655
B-49	0.00	1.80	0.1	0.0	1.000
BW50	3.92	1.40	0.7	2.8	0.428
B-51	0.00	9.80	4.4	0.0	0.036
BW52	1.96	1.12	0.0	1.7	1.000
BW53	0.00	0.72	0.0	0.0	1.000
BW55	4.00	4.12	0.1	0.9	0.752
BW56	2.00	0.74	0.0	2.7	1.000
BW57	7.84	6.56	0.0	1.2	1.000
BW58	1.96	1.06	0.0	1.8	1.000
BW60	11.76	10.58	0.0	1.1	1.000
BW61	0.00	1.65	0.1	0.0	0.752
BW62	23.52	15.14	2.1	1.7	0.147
BW63	0.00	1.16	0.0	0.0	1.000
BW70	0.00	0.90	0.0	0.0	1.000
BW71	0.00	0.37	0.5	0.0	0.479
BW72	0.00	0.50	0.2	0.0	0.655
BW-4	48.00	57.88	1.5	0.6	0.221
BW-6	98.03	88.84	3.4	6.3	0.065
CW-1	5.88	6.18	0.0	0.9	1.000
CW-2	5.88	10.33	0.6	0.5	0.438
CW-3	30.00	30.24	0.0	0.9	1.000
CW-4	27.45	19.67	1.4	1.5	0.237
CW-6	20.00	18.48	0.0	1.1	1.000
DR-1	23.52	21.79	0.0	1.1	1.000
DR-2	22.00	27.41	0.4	0.7	0.527
DR-3	27.45	24.83	0.0	1.1	1.000
DR-4	25.49	28.17	0.0	0.8	1.000
DR-5	19.60	20.99	0.0	0.9	1.000
DRW-6	29.41	30.97	0.0	0.9	1.000
DR-7	23.52	20.21	0.1	1.2	0.752
DRW-8	5.88	5.61	0.1	1.0	0.752
DRW-9	3.92	2.38	0.0	1.6	0.584
DRW10	1.96	2.31	0.0	0.8	1.000
DRW11	15.68	14.82	0.0	1.0	1.000
DRW12	3.92	4.51	0.0	0.8	1.000
DRW13	0.00	2.98	0.3	0.0	0.584
DRW52	70.58	68.53	0.0	1.1	1.000
DRW53	45.09	45.99	0.0	0.9	1.000
DQW-1	60.78	69.40	1.3	0.6	0.254
DQW-2	41.17	37.34	0.1	1.1	0.752
DQW-3	43.75	47.37	0.1	0.8	0.752

The control group consisted of 2495 healthy blood donors in Amsterdam.

HLA-A, HLA-B, and HLA-C typing on lymphocytes was performed by the standard National Institutes of Health (NIH) two stage microcytotoxicity test and HLA-DR and HLA-DQ typing by the two colour fluorescence test.

The antigen frequencies were calculated by

direct counting. Frequencies were compared with the standard panel of the Central Laboratory of The Netherlands Red Cross Blood Transfusion Service in Amsterdam. Statistical significance between the relative frequencies of antigens in patients and controls was determined by χ^2 and Fisher's exact tests with Yates's correction. Exact p values were corrected for 80 informative comparisons, because one can expect to find, by chance alone, four to five antigens significantly increased or decreased (type I or α error). The correction is performed by multiplying the exact p value with the number of antigen frequencies tested (in our study, $\times 80$). The level of significance is then evaluated according to the following scheme: $0.05 > p > 0.01$ is probably significant; $0.01 > p > 0.001$ is significant; and $p < 0.0001$ is highly significant.

The diagnosis of ocular toxoplasmosis was based on the clinical picture of focal chorioretinitis and typical appearance of atrophic pigmented scars.⁴ All patients were positive for circulating anti-toxoplasma antibodies. All patients from group 1 received an aetiological screening for uveitis including HLA-B27 typing, serological tests for syphilis, serum angiotensin converting enzyme, serum lysozyme, and chest x rays. All results were within normal range. The diagnosis of severe congenital toxoplasmosis of the patients in group 2 was based on seroconversion of the mothers during pregnancy (three patients), persisting positive anti-toxoplasma titres of the neonates (six patients) and the clinical presentation with ocular and neurological symptoms in all 10 patients. The diagnosis of the neurological disease (group 2) was based on the clinical picture: all had psychomotor retardation and epilepsy. Six of the 10 patients with severe congenital toxoplasmosis had intracranial calcifications and eight of the patients had a microcephalus or hydrocephalus.

To determine the severity of the ocular disease, all 52 patients were subdivided according to unilateral or bilateral involvement and peripheral or macular localisation of the scar.

Results

HLA antigen frequencies of the mothers of patients with ocular toxoplasmosis and controls are given in Table 1; no specific HLA antigen association was observed in the group of mothers of patients with ocular toxoplasmosis compared with the controls after correction of the p values for the number of antigens tested.

We found a total absence of the HLA-B51 antigen in 47 mothers of patients with ocular toxoplasmosis (groups 1 and 2). The frequency of the HLA-B51 antigen in the control group is approximately 10% (exact p value 0.036). Calculation of the sample size needed to obtain significant results after correction for the number of antigens tested indicated that we would have had to test approximately 150 mothers of patients with ocular toxoplasmosis and that all the tested individuals should also

Table 2 Selected HLA phenotype frequencies in patients with ocular toxoplasmosis

Antigen	Antigen frequency		χ^2	Exact p value	Relative risk
	Patients (%) (n=52)	Controls (%) (n=2495)			
HLA-B15	28.84	16.68	4.54	0.033	2.02
HLA-Bw62	26.92	15.32	4.39	0.036	2.03

Table 3 Selected HLA antigen frequencies in consecutive patients with ocular toxoplasmosis without neurological symptoms

Antigen	Antigen frequency		χ^2	Exact p value	Relative risk
	Patients (%) (n=42)	Controls (%) (n=2495)			
HLA-B7	11.90	26.95	4.04	0.044	0.336

Table 4 HLA-Bw62 frequencies in three subgroups of patients with ocular toxoplasmosis

Antigen	HLA-Bw62 frequency			Exact p value	Relative risk
	Patients (%)	Controls (%) (n=2495)	χ^2		
Severe congenital toxoplasmosis (n=10)	50.00	15.32	6.71	0.009	2.48
Bilateral ocular involvement (n=24)	37.50	15.32	7.32	0.007	3.32
Macular toxoplasmic scars (n=36)	33.33	15.32	7.46	0.006	2.76

be HLA-B51 negative. The frequency of the HLA-A9 antigen in the group of mothers was 8% compared with 20% in the control group (exact p value 0.043). The HLA-Aw19 antigen was found in 35% of the mothers of patients with ocular toxoplasmosis, an increased frequency in comparison with the frequency in the control group of 22% (exact p value 0.043). After correction of the exact p values for the number of HLA antigens tested (80), the observed differences levelled out for all comparisons.

Selected HLA antigen frequencies in the 52 patients with ocular toxoplasmosis (group 1 and group 2) are given in Table 2. No association between any specific HLA antigen and ocular toxoplasmosis was observed. The HLA-B15 antigen and particularly the subtype HLA-Bw62 antigen showed an increased frequency when compared with controls, and was associated with a low relative risk (Table 2).

In patients with ocular toxoplasmosis, but without neurological symptoms (group 1), the frequency of the HLA-B7 antigen was decreased with a low relative risk (Table 3). In 10 patients with severe congenital toxoplasmosis including neurological abnormalities (group 2), an increased frequency of the HLA-Bw62 antigen was observed (Table 4).

When all the 52 patients were subdivided according to (1) unilateral or bilateral ocular involvement (14 patients of group 1 and all the 10 patients of group 2 had bilateral ocular involvement); and (2) the central or peripheral localisation of the retinal scars (macular localised toxoplasmic scars were present in 26 patients of group 1 and in all the patients of group 2). We observed an increased frequency of the HLA-Bw62 antigen in patients

with bilateral ocular involvement ($p=0.007$, Table 4) and with macular localisation of the toxoplasmic scars ($p=0.006$, Table 4). Furthermore, an association of the HLA-Bw62 antigen and severe congenital toxoplasmosis was noted ($p=0.009$, Table 4). After correction of the exact p values for the number of HLA antigens tested (80), the observed deviations did not reach a level of significance.

Discussion

In our study we found no specific HLA antigen phenotype association in 47 mothers of patients with ocular toxoplasmosis, predisposing for developing congenital toxoplasmosis in their of offspring.

The absence of maternal immunogenetic factors in the development of toxoplasmosis in the offspring may be due to confounding factors in the study design employed. The main factors determining maternal-fetal transmission of infection and severity of the clinical manifestations are dependent on the gestation.^{6,7} The chance of transplacental infection increases by trimester. The study design as performed here did not take these issues into account since the gestation at which primary maternal infection and the subsequent fetal infection took place are extremely difficult to measure in a retrospective study. Furthermore it cannot be excluded that some of the cases may have had acquired rather than congenital infection. Current estimates of ocular toxoplasmosis in the Netherlands, however, indicate that less than 5% of cases are expected to be acquired as based on serological analysis.¹³

We observed an absence of the HLA-B51 antigen in 47 mothers of ocular toxoplasmosis patients, which is in accordance with earlier studies of Bertrams and colleagues who reported a decreased frequency of the HLA-B5 antigen in 48 chorioretinitis patients compared with 1000 controls.¹⁴ The current data did not reach statistical significance since in this type of study whereby a considerable number of antigen frequencies are compared between patients and controls the obtained p values are multiplied by the number of antigens tested to compensate for the so called type I or α error.

The HLA-B51 antigen is a part of the HLA-B5 complex. In 1973 an association between Behçet's disease and the presence of the HLA-B5 antigen was found.⁹ The absence of the HLA-B51 antigen could suggest a protective function of the antigen, but clearly further studies are needed to clarify this phenomenon. At least two genes, one within the major histocompatibility complex, have been identified in a murine model of toxoplasmosis, which strengthen our hypothesis that immunogenetic factors are involved in this infectious disease.¹⁵

An increased frequency of the HLA-Bw62 antigen, a subtype of the HLA-B15 antigen, was observed in patients with severe congenital toxoplasmosis and in patients with severe bilateral ocular involvement with macular

localisation of scars. It seems that severe ocular involvement in congenital toxoplasmosis may be associated with the HLA-Bw62 antigen.

Earlier studies by Nussenblatt *et al* who performed HLA typing in 38 ocular toxoplasmosis patients reported a two to threefold increased frequencies of the HLA-A29, Cw7, Dr3, Drw8, Drw52, Dqw1, and Dqw2 antigens, which did not appear to be of clinical or prognostic value according to the authors.¹¹ In the latter study no correction was made for the number of HLA antigens investigated to compensate for the type I error in the statistical analysis.

In conclusion, we did not find a positive HLA antigen association in mothers of patients with ocular toxoplasmosis for developing congenital toxoplasmosis in their offspring. Neither did we find an association of the patients with ocular toxoplasmosis with any HLA-antigen, except a possible association of the severity of ocular involvement and the presence of the HLA-Bw62 antigen.

At present, no precautionary measures based on HLA typing either in pregnant women or in patients with ocular toxoplasmosis can be recommended.

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