Relationship between unexplained infertility and human leukocyte antigens and expression of circulating autogeneic and allogeneic antisperm antibodies

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Summary

Purpose: The association between unexplained infertility, human leukocyte antigens (HLA) and expression of circulating antisperm antibodies was studied in 52 couples with unexplained infertility and 15 infertile and 9 fertile couples.

Methodology: Evaluation parameters included Terasaki's HLA microlymphocytotoxicity test, circulating antisperm antibodies using immunofluorescence technique.

Results: Fifty-two couples (8.7%) out of 600 consecutive clinic attendants had unexplained infertility. Unexplained infertility was associated with circulating antisperm antibodies (22 versus 13 and 0% for men and 18.5 versus 13 and 0% for women). HLA class 1 B_6 and B_{52} and C_{w7} and HLA class 2 DR_4 and DR_6 and sharing of HLA B_6, DR_4 and DR_6 were found more in couples with unexplained infertility. Combined expression of antisperm antibodies by couples, demonstrated more homozygosity for HLA B_6 and DR_4.

Conclusion: Homozygosity for these antigens, B_6, DR_4 and DR_6, may enhance the expression of antisperm antibodies, and cause infertility.

Introduction

Unexplained infertility (UI) is a term used for couples where the cause of infertility is unknown despite a thorough clinical evaluation with standard techniques. Review of the medical literature reveals an incidence of U.I. ranging from 10-30% with an average of about 15%. The discrepancy in the incidence may be attributed to differences in population characteristics, variability in investigative protocols, and availability of chemical and diagnostic facilities [1]; the cause of infertility may be diverse and the number of observations have been small [2]. For nearly 30 years, there have been sporadic reports of HLA associations with neural tube defects, spontaneous abortions and infertility [3]. Many studies have both supported and refuted an association between HLA antigens and reproductive performance. The authors emphasized that the controversy involving association of HLA and reproductive performance can be explained by properly classifying recurrent spontaneous abortions and unexplained infertility [4]. It is well accepted that maternal recognition of paternally-derived fetal antigens occurs during normal pregnancy and may be beneficial for implantation and maintenance of gestation.

Major histocompatability differences between mother and fetus may facilitate implantation and maintenance of pregnancy; and some cases of unsuccessful embryo tranfer (ETs) after in vitro fertilization (IVF) might be caused by underlying close histocompatibility between partners [5] in that couples who share multiple HLA antigens may have an immunologic basis for failing to achieve a viable pregnancy with multiple assisted reproductive technique (ART) cycles. There is evidence of a significant excess of HLA-DR sharing in couples with RSA and a significant excess of HLA-DQ sharing in couples with unexplained infertility who failed treatment by IVF [6]. There are recent reports that sharing of human leucocyte antigens in couples with unexplained infertility affects the success of IVF and tubal EF, and they are located in the B-DR-DQ region of the major histocompatability complex [7]. Support for the influence of HLA on fertility is provided by a prospective study in Hutterites by Ober *et al.* [8] that showed a marked decrease in fertility in couples sharing HLA-DR antigen.

Objective of study

To investigate the relationship between HLA classes I and II and unexplained infertility and the association with circulating antisperm antibodies.

Central Hypothesis

HLA class I and II regions are associated with unexplained infertility as a result of antisperm antibodies.

Patients and Methods

During a four-year-period, 600 couples were seen at the combined infertility clinic at the Maternity Hospital, Kuwait. Fifty-two couples who went through the infertility evaluation protocol of the unit form the subjects of the present study. Fifteen other couples with known aetiological factors for infertility and

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nine fertile couples formed the control groups. Inclusion criteria for the study group were couples without any detectable abnormality using the standard methods of patient evaluation. All patients with uterine anatomical abnormalities like septate and bicornuate uterus, anovulation, endometriosis, azoospermia, oligozoospermia, asthenozoospermia and necrozoospermia were excluded from the study. Before inclusion into the study however, patient consent was obtained.

The evaluation protocol included a detailed history and physical evaluation, semen analysis, antisperm antibodies, hormone profile-FSH, LH, prolactin, testosterone and mid-luteal phase progesterone for the female spouse and thyroid function tests. In this study, patients with circulating antisperm antibodies were included in the study group although they were later analysed separately and compared with those without, but who had unexplained infertility. Two control groups of infertile couples with known aetiology for infertility and a group of fertile couples were used. Couples in the study and control groups had assays for circulating antisperm antibodies with the immunofluorescence technique and HLA phenotyping using the modified Terasaki microlymphocytotoxicity test as previously described [9]. While the former used sera stored at -20°C, the latter used fresh whole blood.

Results

The characteristics of the study and control couples are summarised in Table 1. About 87% of the couples with unexplained infertility presented with primary infertility.

Taking the cut-off antisperm antibody titre of 1:128 as positive (Table 2) 23.1% of men in an unexplained infertility union compared to 13% of men in unions with known infertility factor ($p<.05$) and none in the fertile controls had antisperm antibodies. Among the women 19.2% of those in unexplained infertility unions had antisperm compared to 13% of those with known causes of infertility ($p<.05$); seven couples in the unexplained infertility group had antisperm antibodies compared to one in the known infertility group.

Table 3 compares the HLA profile of men and women with the known infertility factor group and the controls. HLA class 1 B_6, B_{52} and C_{w7}, and DR_4 and DR_6 were more significant expressed in the unexplained infertility than the known infertility factor group ($p<.0.05$, 0.05, 0.05, 0.05 and 0.05).

Among the women HLA class I - B_6, B52 and Cw7 and Class DR_4 and DR_6 are more highly expressed in the unexplained infertility group than in the known infertility factor group and the controls.

Table 4 evaluates the association between sharing of HLA haplotypes and unexplained infertility among couples. HLA antigens B_6, DR_4 and DR_6 were more highly expressed in the unexplained infertility group than the others. Conversely, DQ_3 was more highly expressed in the known infertility group and the fertile controls than in the unexplained infertility group.

In order to test the hypothesis that HLA sharing is associated with antisperm antibodies in unexplained infertility we decided to evaluate the effect of circulating antisperm antibodies on the HLA frequency occurrence and the HLA homozygosity. As shown in Table 5, men and women with circulating antisperm antibodies expressed HLA, B_6, B_{52}, DR_4 and DR_6 more than those without antisperm antibodies. Secondly, in unions where both couples had circulating antisperm antibodies, they were more likely to show homozygosity of B_6, DR_4 and DR_6.

Table 1. — *Characteristics of the study and control couples.*

	Unexplained Infertility n = 52	Known cause of Infertility n = 15	Controls n = 9
Duration of marriage (years)	5.4±2.4	7.2±4.1	5.1±2.6
Duration of infertility (years)	4.0±2.1	5.6±3.8	–
Primary infertility (%)	45 (87)	11 (73)	–
Secondary infertility (%)	7 (13)	4 (27)	–
Mean parity	0.2±0.1	0.4±0.4	2.4±0.8

Table 2. — *Incidence of circulating antisperm antibodies.*

	Unexplained Infertility n = 52	Known infertility factor n = 15	Controls n = 9
Men			
ASA titres			
1:128	2	–	–
1:256	4	–	–
1:512	5	1	–
>1:512	1	1	–
Total	12	2	0
Percent	23.1	13	0
Women			
ASA titres			
1:128	4	–	–
1:256	2	–	–
1:512	1	2	–
>1:512	3	–	–
Total	10	2	0
Percent	19.2	13	0
ASA in couples	9	1	0

ASA: Antisperm antibodies

Discussion

The main finding of this study was that unexplained infertility is associated with the HLA-B-DR region, and that this may be mainly due to the occurrence of antisperm antibodies. In couples with unexplained infertility with antisperm antibodies, HLA B_6, DR_4, DR_6 were more common than in couples with unexplained infertility without antisperm antibodies. Our observation is partially in agreement with a previous study of HLA antigens and infertility with sperm autoantibodies (SAA) in men. Although there was no prevalence of HLA-DR Ag and infertility with SAA, HLA-A, B Ag were more frequently observed in the group with SAA [10]. Taylor and associates [11] have reported higher autoantibodies, smooth muscle antibody, antinuclear antibody and antiphospholipid antibody in couples with unexplained infertility than in controls. It has been estimated that 71% of women with unexplained infertility showed evidence of autoantibodies aPL, antithyroid, systemic $CD56^{+cells}$ and serum embryo-

Table 3. — *HLA profile of men and women*

	Unexplained Infertility n = 52	Known infertility factor n = 15	Controls n = 9
HLA			
Men			
A_1	5 (9.6)	2 (13.3)	1 (11.1)
A_{11}	3 (5.8)	1 (6.7)	1 (11.1)
B_6	12 (23.1)	1 (6.7)	0 (0)
B_{w8}	4 (7.7)	2 (13.3)	2 (22.1)
B_{s2}	11 (21.2)	1 (6.7)	1 (11.1)
C_{w4}	3 (5.8)	1 (6.7)	0 (0)
C_{w7}	8 (15.4)	1 (6.7)	1 (11.1)
C_{w46}	7 (13.5)	3 (20.0)	2 (22.2)
DR_1	2 (3.9)	4 (26.7)	3 (33.3)
DR_4	12 (28.9)	2 (13.3)	1 (11.1)
DR_5	5 (9.6)	2 (13.3)	1 (11.1)
DR_6	8 (15.4)	1 (6.7)	2 (22.2)
DR_{w52}	4 (7.7)	1 (6.7)	0 (0)
DQ_3	8 (15.4)	3 (20.0)	2 (22.2)
DQ_7	6 (11.5)	2 (13.3)	1 (11.1)
Women			
A_1	4 (7.7)	2 (13.3)	0 (0)
A_{11}	3 (5.8)	1 (6.7)	0 (0)
B_6	9 (17.3)	3 (20.0)	1 (11.1)
B_{w8}	2 (3.9)	0 (0)	2 (22.2)
B_{52}	13 (25.0)	1 (6.7)	1 (11.1)
C_{w4}	4 (7.7)	2 (13.3)	1 (11.1)
C_{w7}	9 (17.3)	1 (6.7)	0 (0)
C_{w46}	4 (7.7)	1 (6.7)	1 (11.1)
DR_1	6 (11.5)	2 (13.3)	1 (11.1)
DR_4	12 (23.1)	1 (6.7)	1 (11.1)
DR_5	8 (15.4)	3(20.0)	2 (22.2)
DR_6	14 (26.9)	2 (13.3)	1 (11.1)
DQ_3	7 (13.5)	3 (20.0)	2 (22.2)
DQ_7	3 (5.8)	2 (13.3)	1 (11.1)

Table 4. — *Sharing of HLA haplotypes among couples.*

	Unexplained Infertility n = 52	Known infertility factor n = 15	Controls n = 9
HLA			
Class 1			
A_1	3 (5.8)	1 (6.7)	0 (0)
B_6	8 (15.4)	1 (6.7)	0 (0)
B_{w8}	2 (3.9)	0 (0)	2 (22.2)
B_{52}	5 (9.6)	1 (6.7)	0 (0)
C_{w4}	2 (3.9)	0 (0)	0 (0)
C_{w7}	4 (7.7)	1 (6.7)	1 (0)
Class 2			
DR_4	10 (19.2)	1 (6.7)	1 (11.1)
DR_5	4 (7.7)	2 (13.3)	1 (11.1)
DR_6	8 (15.4)	1 (6.7)	0 (0)
DQ_3	6 (11.5)	3 (20.0)	2 (22.2)

toxic factors [12]. Sperm autoimmunity, especially with circulating antisperm antibodies, have been directly implicated in the pathogenesis of a subset of patients with infertility because of adverse effects on a couple's ability to achieve fertilization [13, 14]. As demonstrated in the present study, antisperm antibodies were increased in subfertile individuals and their presence was associated with

Table 5. — *Effect of circulating ASA on frequency and sharing of HLA.*

	HLA Profile				HLA Sharing	
	A_1 n = 12	A_2 n = 40	B_1 n + 10	B_2 n = 42	C_1 n = 9	C_2 n = 43
B_6	5 (41.7)	7 (17.6)	3 (20.0)	6 (14.3)	4 (44.4)	5 (11.6)
B_{52}	4 (33.3)	7 (17.6)	4 (40)	9 (21.4)	2 (22.2)	3 (7.0)
C_{w7}	2 (16.7)	5 (25.0)	2 (20)	7 (16.7)	1 (11.1)	3 (7.0)
DR_4	6 (50)	9 (22.5)	4 (40)	8 (19.1)	4 (44.4)	6 (14.0)
DR_6	6 (25.0)	5 (12.0)	50 (50)	9 (21.4)	3 (33)	5 (11.6)
DQ_3	2 (16.6)	5 (25.0)	2 (20)	5 (11.9)	1 (11.1)	5 (11.6)

( ) Percent:
A_1 = Men with antisperm antibodies;
A_2 = Men without anti-sperm antibodies;
B_1 = Women with antisperm antibodies;
B_2 = Women without antisperm antibodies;
C_1 = Both couple express antisperm antibodies;
C_2 = Couples without antisperm antibodies.

decreased fecundity [15]. Identification of this cohort of patients with unexplained infertility is necessary because immunosuppression has been shown to improve the sperm quality and conception rates [14]. The critical genes or genetic defects that significantly affect the success of invitro fertilization and tubal embryo transfer are located in the B-DR-DQ region of the major histocompatability complex [7]. The mechanism of an adverse effect of HLA on fertilisation may be a result of antisperm antibodies, which as an autoimmune entity, is controlled by HLA. Human leucocyte antigen systems appear to also be involved in the genesis of anti-phospholipid syndrome, another autoimmune condition with high pregnancy loss as a consequence of widespread abnormality of early uteroplacental vasculopathy, with platelet aggregation and placental infarction [11]. Antisperm antibodies may thus affect early pregnancy directly in a similar fashion, or by association with other deleterious autoantibodies. Furthermore, 85% of all pregnancy wastage occurs before clinical awareness of pregnancy. Women with recurrence of preclinical pregnancy failures have unexplained infertility rather than recurrent spontaneous abortion; two conditions believed to be manifestations of a common underlying pathologic condition [16], autoimmunity.

In conclusion, homozygosity for the antigens D_6, DR_4 and DR_6 may enhance the expression of antisperm antibodies and cause infertility.

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