ELSEVIER SCIENTIFIC PUBLISHERS IRELAND

Journal of Reproductive Immunology 25 (1993) 195-207

JOURNAL OF REPRODUCTIVE IMMUNOLOGY

MHC class II compatibility in aborted fetuses and term infants of couples with recurrent spontaneous abortion

Carole Ober [*a'b], Thomas Steck [a], Katrin van der Ven [a], Christine Billstrand [a], Lori Messer [a], Joanne Kwak`, Kenneth Beaman`, Alan Beer`

"Molecular Genetics Laboratory, Department of Obstetrics and Gynecology, [t]he Committee on Evolutionary Biology, The University of Chicago, MC2050, 5841 Maryland Avenue. Chicago, IL 60637. USA `Department of Microbiology and Immunology. University of Health Sciences/Chicago Medical School. 3333 Green Bay Road. North Chicago. IL 60064, USA

(Accepted 7 October *1993)*

Abstract

Maternal-fetal histocompatibility for alleles at HLA class II loci, *HLA-DQAI* and *HLADQBI,* was examined in 40 abortuses and 31 liveborn children of 68 couples with a history of idiopathic recurrent spontaneous abortion (RSAB) who underwent leukocyte immunization prior to the index pregnancy. Significantly more couples with RSAB shared two *HLADQAI* alleles as compared with fertile control couples (0.18 vs. 0.03, respectively; $P = 0.031$). There were no differences in HLA sharing between couples with RSAB who experienced a repeat abortion in the index pregnancy as compared with couples with RSAB who were delivered of a liveborn child. Non-significant deficits of abortuses who were compatible for alleles at the *HLA-DQAI* (6 observed vs. 8.5 expected; P=0.225) and the *HLA-DQBI (7* observed vs. 9.2 expected; P=0.254) loci were observed. A significant deficit of *HLA-DQAI* compatible liveborn children was observed (I observed vs. 5.5 expected; P = 0.0069). The overall deficit of *HLA-DQAI* compatible fetuses (7 observed vs. 14.0 expected; P=0.0018) after approximately 8 weeks gestation suggests that *HLA-DQAI* compatible fetuses may be aborted early in pregnancy, prior to the time when fetal tissue can be recovered for genetic studies.

Key words: HLA sharing; *HLA-DQAI;* Recurrent spontaneous abortion

* Corresponding author.

 SSDI 0165-0378(93)00856-0

1. Introduction

Maternal immunologic recognition of the fetal allograft has long been recognized as a consequence of normal pregnancy (Payne and Rolfs, 1958; Van Rood et al., 1958). Mothers are sensitized to paternal HLA in nearly half of multiparous pregnancies without any ostensibly deleterious effects (Larson, 1983). Because recognition of paternally derived HLA occurs in normal pregnancy but only rarely in women with recurrent miscarriage, it was proposed that maternal recognition of the fetal allograft may actually be beneficial and that fetal loss may result from the maternal immune system failing to recognize paternally derived HLA in histocompatible gestations (Beer and Billingham, 1976; Gill and Repetti, 1979; McIntyre and Faulk, 1982).

If this hypothesis was true, then couples experiencing idiopathic recurrent miscarriage may share HLA more often than fertile couples, because only couples sharing HLA can produce compatible fetuses. Increased HLA sharing among couples experiencing recurrent abortion as compared with fertile control couples was first reported in 1979 (Komlos et al., 1979; Schacter et al., 1979). Although these early studies supported a role for HLA sharing in recurrent miscarriage, numerous subsequent studies have yielded conflicting results regarding associations between HLA sharing and recurrent miscarriage (reviewed by Ober, 1992). Discrepancies between studies have been difficult to reconcile because of differences in the selection criteria for couples with recurrent miscarriage as well as in the choice and definition of 'control' subjects.

In addition to differences in sample definition, measuring HLA sharing between partners does not provide unambiguous information on the genetic makeup of the fetus. No studies to date have directly tested the hypothesis that HLA compatible fetuses are at a selective disadvantage during pregnancy. To our knowledge, this is the first study to HLA type aborted fetuses of couples with histories of recurrent spontaneous abortion (RSAB). In this report we present results of studies of maternal-fetal compatibility with respect to the class II genes, *HLA-DQAI* and *HLA-DQBI*.

2. Material and methods

2.1. Sample composition

Beginning July 1989, all couples undergoing paternal leukocyte immunization as treatment for recurrent miscarriage at Chicago Medical School (Beer, 1988) were instructed to send parents' blood samples and placental tissue to the Molecular Genetics Laboratory at the University of Chicago, if a spontaneous abortion occurred in the next pregnancy.

Between October 1989 and June 1992, placental specimens from 106 abortuses and parents' blood samples were received in the laboratory. Specimens were examined under a dissecting microscope to identify fetal tissue (villi). Maternal decidual tissue was removed using fine forceps and villi was cleaned with Hanks balanced salt solution. Fetal tissue was identified in 62 specimens; only maternal tissue (decidua) was present in the remaining 44 specimens. Among the 62 specimens in which fetal tissue was identified, 21 were excluded from analysis for the following reasons: 7 abortuses were chromosomally abnormal, 5 had abnormal ultrasound findings prior to abortion but cytogenetic studies were unsuccessful, 8 were either ectopic pregnancies, fetal demises at > 28 weeks gestation, or elective abortions and 1 was from a subject who became pregnant prior to paternal leukocyte immunization. After DNA studies were completed, one additional abortus was excluded from the analysis because no paternal alleles were identified in the sample suggesting that the sample contained maternal and not fetal, DNA. The presence of paternal DNA was confirmed in the remaining 40 samples.

The 40 abortuses were from 37 couples with three or more losses prior to the index pregnancy, who aborted following immunization with paternal leukocytes. Ten couples had one and three couples had two previous liveborn children. All other genetic, endocrinologic, anatomic and autoimmunologic causes of miscarriage had been excluded in these couples prior to the index pregnancy. Normal cytogenetic studies were reported in 19 abortuses (study group 1), but cytogenetic studies were not performed in 21 (study group 2).

Two control groups were used for comparisons. The first included 31 children of 31 couples with successful pregnancies following paternal leukocyte immunization at Chicago Medical School (study group 3). These couples had three or more losses prior to the index pregnancy. Genetic, endocrinologic, anatomic and autoimmunologic causes of miscarriage had been excluded in these couples prior to the index pregnancy. These couples were identified retrospectively and asked to participate in this study by providing a blood sample (from the parents) or buccal smear (children) for DNA studies. Because of the limited amount of DNA obtained from buccal smears, these families were typed for alleles at the *HLA-DQA1* locus only.

A second control group included 36 couples without a history of miscarriage and at least one liveborn child. One child from each of 20 couples was typed so that parental haplotypes could be determined. These families participated in other studies in our laboratory (Van der Ven et al., 1992; Ober et al., 1993).

2.2. Laboratory methods

DNA was extracted from 5-10 ml blood from parents and older children and from at least 10 ug chorionic villi in abortuses (Bell et al., 1981; Boehm

et al., 1983) and resuspended in 0.l x TE buffer (1 mM Tris-HCI, 0.l mM EDTA, pH 8.0). DNA was obtained from buccal smears in 24 infants delivered following immunotherapy (Higuchi, 1989). The second exon of the *HLA-DQAI* gene was amplified by the polymerase chain reaction (PCR) as follows: 0.5-1 µg DNA was subjected to 30 cycles of PCR in 100 µl buffer (10 mM Tris (pH 8.3), 50 mM KCl, l.5 $_{MM}$ $MgCl_2$) containing 3 units TaqDNA polymerase (Perkin-Elmer-Cetus, Norwalk, CT) and 50-100 pmol of each of primers GH26 and GH27 (Gyllensten and Erlich, 1988), as described previously (Saiki et al., 1986). Each PCR cycle consisted of 2 min denaturation at 94°C, 2 min annealing at 37°C and 2 min extension at 72°C in a DNA thermal cycler (Cetus); the first cycle was preceded by a 3.5 min denaturation at 94°C and the last cycle was followed by a 7 min extension phase at 72°C. The second exon of the *HLA-DQBI* gene was amplified by PCR for 35 cycles (1 min at 94°C, 30 s at 60°C, 30 s at 72°C), preceded by 6 min at 94°C and followed by 7 min at 72°C, using 50-100 pmol of each primer DQB-3 and DBl30 (Bugawan and Erlich, 1991). DQB-3(5'CTGGTAGTTGTGTCTGCACAC-3') is a shortened version of GH 29 (Horn et al., 1988). The quantity of the PCR product was determined by electrophoresing 18 µg of the PCR product on an agarose gel and by hybridization with probes that hybridize to all *DQAI* alleles or to all *DQBI* alleles.

A 5-10-µl aliquot of the PCR reaction was denatured in 0.4 N NaOH at 98°C for 2 min, added to 0.2x SSC (30 mM NaCI, 3 mM sodium citrate) and blotted by vacuum onto nylon. membranes (Gene Screen", NEN, Boston, MA). The amplified DNA was immobilized by air drying overnight or by baking at 80°C for 30 min. The sequences of the 10 DQAI sequencespecific oligonucleotide probes (SSOPs) and 13 DQBI SSOPs are reported elsewhere (Erlich and Bugawan 1989; Helmuth et al., 1990; Nelson et al., 1993). SSOPs were labeled with y-[^{32} P]ATP at their 5'-ends by polynucleotide-kinase; unincorporated nucleotide was removed by centrifugation in a G-25 Sephadex Quick Spin' column according to the manufacturer's instructions (Boehringer Mannheim, Indianapolis, IN). Blots were prehybridized for 2 h at 42°C in 50 mM $NaPO_4$ (pH 6.5), 0.5 M NaCl. l% sodium dodecyl sulfate (SDS), 5 x Denhardt's (0.1 Ficoll, 0.1 polyvinylpyrrolidone, 0.l% bovine serum albumine) and 200µg/ml sonicated and denatured salmon sperm DNA in a shaking incubator. SSOPs were hybridized to blots for 12 h using the same buffer plus 0.5-l x 10^6 counts
per min/ml labeled SSOPs. The membranes were washed twice in 5 x SSC, l% SDS at room temperature and twice in 2 x SSC, 1 SDS at 51-63°C, depending on the sequency of the probe. Membranes were exposed to autoradiographic films (X-Omat AR', Kodak, Rochester, NY) for l-4 h. SSOPs were stripped from blots by washing in 0.4 N NaOH for 30 min at 42°C and in 0.l x SSC, 0.l SDS, 0.2 M Tris-HCI (pH 7.5) for 30 min at

42°C. Blots were reused up to four times without significant loss of bound DNA.

HLA-DQAI and *HLA-DQBI* alleles were designated according to the nomenclature provided by the 1991 report of the WHO HLA Nomenclature Committee (Bodmer et al., 1991). DQAI/DQBI haplotypes were determined by direct observation of segregating alleles in parents and abortuses or liveborn children (in control couples).

3.3. Statistical analyses

The proportions of couples sharing HLA were compared using contingency table X' analysis.

The expected numbers of compatible or homozygous fetuses were calculated assuming random segregation. For couples sharing one allele at a locus, the expected frequency of compatible fetuses is 0.50 and the expected number of homozygous fetuses is 0.25. For couples sharing two alleles at a locus, the expected frequency of compatible fetuses is l.0 and the expected number of homozygous fetuses is 0.50. For couples in whom the mother is homozygous for an allele that her partner is heterozygous for, the expected frequency of compatible fetuses is 0.50 and the expected number of homozygous fetuses is l.0 (i.e.., all compatible fetuses will be homozygous). For couples in whom the father is homozygous for an allele that the mother is heterozygous for, the expected frequency of compatible fetuses is 1.0 and the expected frequency of homozygous fetuses is 0.50. Expected and observed numbers were compared using the $_{X \text{ square}}$ goodness-of-fit test when the number of expecteds was greater than 5. For comparisons that included cells with expected values of less than 5, the data are presented but statistical tests were not performed because the power to detect differences in these small samples was not sufficient to warrant the calculation of P values.

3. **Results**

The mean ages, mean gestational ages of abortuses, mean number of previous abortions and the proportion of couples with a previous liveborn are shown in Table 1 for couples with RSAB in group I (cytogenetically normal abortuses), group 2 (cytogenetic status of abortuses unknown) and group 3 (livebirths). The three groups were similar with respect to the variables shown.

3.1. Class 11 sharing in couples with recurrent SAB and in fertile controls

The HLA sharing status of couples with recurrent SAB (groups 1, 2 and 3) as compared with fertile controls is shown in Table 2. There were no differences in *HLA-DQAI* allele sharing among couples experiencing a repeat

Table I
Characteristics of study population. Couples with three or more unexplained abortions prior to the index pregnancy

	Mean maternal age (years) (Range)	Mean gestational age (weeks) (Range)	Mean no. prey. SABs (Range)	Prop.. couples with previous Liveborn
Group 1	32 (27-39)	12 (8-25)	4.7 (3-8)	0.29
Group 2	34 (28-43)	10 (6-17)	4.4 (3-10)	0.35
Groups I + 2	33 (27-43)	11 (6-25)	4.5 (3-10)	0.33
Group 3	32 (23-43)		4.3 (3-11)	0.28

Group I, abortuses with normal cytogenetic studies (19 abortuses of 17 couples). Group 2. abortuses in which cytogenetic studies were not performed (21 abortuses of 20 couples). Group 3, liveborn children (31 couples). All pregnancies were conceived following paternal leukocyte immunization (Beer, 1988).

abortion in the index pregnancy (groups I and 2) as compared with couples delivered of a liveborn child in the index pregnancy (group 3) (P = 0.267). The proportion of couples sharing 0, 1, or 2 *HLA-DQAI* alleles was not significantly different among couples with RSAB (groups l, 2 and 3) as compared with fertile controls (P = 0.081). However, the proportion of couples sharing two *HLA-DQAI* alleles was significantly increased in couples with RSAB as compared with fertile couples (0.18 vs. 0.03, respectively; $P = 0.029$). There were no differences in *HLA-DQBI* allele sharing or *HLADQAI/DQBI* haplotype sharing among couples with recurrent SAB (groups 1 and 2) as compared with fertile controls (P = 0.471 and 0.682, respectively).

3.2. Class II compatibility in fetuses of couples sharing one allele or haplotype

All conceptuses of couples not sharing alleles or haplotypes will be incompatible with respect to that locus or haplotype and all conceptuses of couples sharing both alleles (or haplotypes) will be compatible with respect to that locus (or haplotype). Fetuses of couples sharing one allele or haplotype can be compatible or incompatible and the expected frequency of compatible and incompatible fetuses can be predicted based on Mendelian (random) segrega-

Table 2
HLA-DQAI and *HLA-DQBI* sharing in couples with SAB and in fertile couples

	No. alleles shared				P value (2 df)
	0	1	2		
HLA-DQAI					
Groups 1 + 2 (N = 37)	18 (0.49)	15 (0.41)	4 (0.11)		
Group 3 (N=31)	12 (0.39)	11 (0.35)	8 (0.26)	1 + 2 vs. 3:	0.267
Groups 1 +2+3 (N = 68)	30 (0.44)	26 (0.38)	12 (0.18)		
Fertile couples (N= 36)	20 (0.55)	15 (0.42)	1 (0.03)	1 + 2 + 3 vs. fertiles:	0.081
HLA-DQBI					
Groups 1 + 2 (N= 37)	20 (0.54)	16 (0.43)	1 (0.03)		
Fertile couples (N = 36)	23 (0.64)	13 (0.36)	0(0)	i + 2 vs. fertiles:	0.471
	No. haplotypes shared				P value (2 df)
	0	1	2		
HLA-DQBI/ HLA-DQAI					
Groups 1 + 2 (N= 37)	23 (0.62)	13 (0.35)	1 (0.03)		
Fertile couples (N= 20)	14 (0.70)	6 (0.30)	0(0)	I + 2 vs. fertiles:	0.682

Couples with SAB are described in Table 1; fertile couples include couples without previous SAB and at least one liveborn child. Groups compared by x^2 analysis.

tion ratios (discussed above in methods). Therefore, fetuses of couples sharing one allele or haplotype were examined to determine whether the frequency of class II compatible abortuses or livebirths differed from predicted frequencies based on random segregation ratios.

The numbers of compatible and incompatible abortuses (groups 1 and 2) were not significantly different from expected numbers for alleles at the *HLA-DQAI* locus ($P = 0.225$), *HLA-DQBI* locus ($P = 0.254$), or for the *HLA-DQAI/DQBI* haplotype ($P = 0.439$), although in all categories there

Table 3
Expected and observed numbers of *HLA-DQAI* and *HLA-DQBI* compatible fetuses in couples sharing one allele or haplotype

Group	No. fetuses in couples sharing I allele	No. observed (expected)		P value
		Compatible	Incompatible	
HLA-DQAI				
1	*1*	5 (5.0)	5 (5.0)	
2	7	1 (3.5)	6 (3.5)	
1+2	1	6(8.5)	11 (8.5)	0.225
3	11	1 (5.5)	10 (5.5)	0.0067
HLA-DQBI				
1	9	4(4.5)	5 (4.5)	
2	10	3 (5.0)	7 (5.0)	
1+2	19	7 (9.5)	12 (9.5)	0.254
HLA-DQAI/HLA-DQBI haplotype				
1	8	3 (4.0)	5 (4.0)	
2	7	3 (3.5)	4 (3.5)	
1+2	15	6 (7.5)	9 (7.5)	0.439

Expected and observed numbers compared by X^2 goodness-of-fit test, I degree of freedom.

Table 4
HLA-DQAI and *HLA-DQBI* homozygosity in compatible fetuses of couples with unexplained recurrent spontaneous abortion who share one or two alleles or haplotypes

Group	No. compatible fetuses	Observed no. fetuses	
		Heterozygous (expected)	Homozygous (expected)
HLA-DQAI			
1+2	10	4 (4.5)	6 (5.5)
3	8	4(4.0)	4 (4.0)
HLA-DQBI			
1+ 2	8	3 (4.0)	5 (4.0)
HLA-DQAI/HLA-DQBI			
1+ 2	6	2 (3.0)	4 (3.0)

were more incompatible than compatible fetuses observed (Table 3). Among 11 liveborn children of couples sharing one *HLA-DQAI* allele, 10 were incompatible with respect to this locus. This was significantly different than the expectation of *5.5* compatible children *(P* = 0.0067).

3.3. Homozygosity and heterozygosity in compatible fetuses of couples who share alleles or haplotypes

Compatible fetuses may be heterozygous (identical to mother) or homozygous for an allele for which the mother has one or two copies. The frequencies of homozygous and heterozygous compatible fetuses are shown in Table 4 for couples sharing 1 or 2 alleles. Proportions of homozygous and heterozygous compatible fetuses appeared similar to expected proportions in abortuses or liveborn children, but P values were not calculated because the sample sizes were too small.

4. Discussion

Prior to the availability of HLA typing by DNA methods, determining the HLA types of aborted fetuses was not practical. As a result, previous studies of HLA sharing in recurrent abortion have focused on parental HLA sharing and assumed that couples with recurrent abortion who share HLA were aborting compatible fetuses. This study was designed to directly test the hypothesis that couples with recurrent abortion. who share HLA were preferentially aborting HLA compatible fetuses. *The class II loci, HLA-DQAI* and *HLA-DQBI,* were studied because increased sharing of HLA-DR among recurrent aborters is commonly reported (Unander and Olding, 1983; Reznikoff-Etievant et al., 1984; Beer et al., 1985; McIntyre et al., 1986) and is particularly characteristic of this patient population (Beer et al., 1985; Beer, 1988).

Non-significant deficits of abortuses that were compatible for alleles at the *HLA-DQAI* locus (6/17), the *HLA-DQBI* locus (7/19) and the *HLADQAI/HLA-DQAI* haplotype (6/15) were observed (Table 3). In addition, a significant deficit of *HLA-DQAI* compatible livebirths was evident (l/11). Thus, in this sample 7/28 fetuses were compatible for *HLA-DQAI* alleles, whereas 14.0 compatible fetuses were expected (P = 0.0067). If the proportion of compatible and incompatible embryos is equal at conception, then the deficiencies of compatible fetuses suggest that compatible fetuses may be lost early in gestation. The mean gestational age of the abortuses in this study was 11.0 weeks and only 13% were less than 8 weeks gestation. Among the abortus specimens received in our laboratory for which fetal tissue could not be identified, the mean gestational age was 7.8 weeks and 45% were less than 8 weeks gestation. Increased frequencies of compatible fetuses among these,

or earlier losses could account for the deficit of compatible fetuses in our study.

Selection against class II compatible fetuses in early gestation has also been suggested by results of prospective studies of pregnancy outcome in a fertile, inbred population, the Hutterites (Ober et al., 1992). Hutterite couples who share HLA-DR take significantly longer to achieve pregnancy than couples not sharing HLA-DR (P = 0.0 16), but do not experience increased recognized fetal loss rates (P = 0.472). The data presented here further suggest that in outbred couples with RSAB, selection against class II compatible fetuses may be occurring prior to 8 weeks gestation. However, even if class II compatible fetuses are at a selective disadvantage in early pregnancy, many survive to term as evidenced by eight compatible livebirths in couples with RSAB who share two *HLA-DQAI* alleles (Table 4). These data further indicate that compatible fetuses that are aborted at 8 weeks or greater or that survive to term are equally likely to have heterozygous or homozygous genotypes (Table 4).

In this investigation, the frequency of HLA sharing was not significantly different among spouses with RSABs who aborted in the index pregnancy (groups 1 and 2) as compared with couples with RSABs who were delivered of a liveborn child (group 3), suggesting that class II allele sharing is not predictive of pregnancy outcome following leukocyte immunization. This finding is similar to other reports that HLA sharing is not predictive of pregnancy outcome in couples undergoing immunotherapy for treatment of RSAB (Cowchock et al. 1990).

It is noteworthy that class II allele sharing was fairly common among fertile controls (42% shared one allele at the *HLA-DQAI* locus, 36% at the *HLA-DQBI* locus and 30% shared one *HLA-DQAI/DQBI* haplotype), although sharing both alleles at a class II locus is infrequent among fertile couples. Only one of 26 control couples shared two *HLA-DQAI* alleles and none shared two *HLA-DQBI.* Sharing two *HLA-DQBI* alleles was also infrequent among couples with SABs; only 1 of 36 couples shared two alleles. In contrast, couples with SABs shared two *HLA-DQAI* alleles significantly more frequently than control couples (P = 0.031). It is unlikely that the increased frequency of sharing two *HLA-DQAI* alleles reflects patient ascertainment biases because during the period of this study couples considered as candidates for immunotherapy were not selected on the basis of HLA sharing and for the majority of couples HLA typing was performed after enrollment into the program. Thus these data suggest that sharing two *HLADQAI* alleles may be a risk factor for SAB. Because *HLA-DQAI* alleles are present on more than one HLA-DR haplotype (e.g.., DQA-1 *0501 is present on both DR3 and DR5 haplotypes), couples sharing *HLA-DQAI* alleles may not always share HLA-DR antigens. Therefore, if the true association be-

tween HLA sharing and RSAB is with alleles at the *HLA-DQAI* locus and not with HLA-DR antigens, then studies of HLA sharing among couples with RSAB that utilize serologic techniques may yield discrepant results. This could explain the inconsistent results in the literature regarding HLADR sharing in couples with RSAB (Ober, 1992). However, proof of this hypothesis requires additional studies of *HLA-DQAI* allele sharing in couples with idiopathic RSAB.

The major objective of this study was to examine the compatibility status of fetuses of couples with SABs. Interpretation of our results is limited by several factors. First, all couples in our study were immunized prior to the index pregnancy and it is not known if leukocyte immunization has equal effects on compatible and incompatible fetuses. For example, the deficits of compatible fetuses observed in this study could be due to immunization preferentially 'rescuing' incompatible fetuses and these results may not be generalizable to fetal losses in untreated aborted pregnancies. Second, the HLA status is not known for the 44 early abortuses in which fetal tissue could not be identified. Therefore, the relationship between maternal-fetal histocompatibility and losses occurring between conception and approx.. 8 weeks gestation is still unknown, although a significant proportion of these early abortuses may be cytogenetically abnormal. Lastly, because the sample sizes (and the corresponding power to detect differences) are small, despite nearly 3 years of data collection, these data should be interpreted cautiously. Nonetheless, this study suggests that sharing two *HLA-DQAI* alleles is associated with recurrent SAB and raises the possibility that selection against class II compatible fetuses occurs in early pregnancy in outbred couples with RSAB. Confirmation of these hypotheses is currently underway in an independent sample of couples with RSAB who are participating in a double-blind, randomized trial designed to evaluate the efficacy of immunotherapy in preventing miscarriage.

5. Acknowledgements

The authors thank Dr. Ann Reed, Michael Hsu and Gary Belen for technical assistance. C.O. is supported by NIH grants HD21244 and HD27686. T.S. was supported by the Deutsche Forschungsgemeinschaft Ste 572/l-I.

6. References

Beer, A.E. (1988) Pregnancy outcome in couples with recurrent abortions following immunological evaluation and therapy. In: Early Pregnancy Loss: Mechanisms and Treatment (Beard, R.W. and Sharp, F., eds.), pp. 337-350. Springer, New York.

Beer, A.E. and Billingham, R.E. (1976) The Immunobiology of Mammalian Reproduction. Englewood Cliffs, NJ, Prentice-Hall.

Beer, A.E., Semprini, A.E., Xiaoyu, Z. and Quebbeman, J.F. (1985) Pregnancy outcome in human couples with recurrent spontaneous abortions: HLA antigen profiles, female serum MLR block factors, and paternal leukocyte immunization. Exp. Clin. Immunogenet. 2, 137-153.

Bell, G.1., Karam, J.H. and Rutter, W.J. (1981) Polymorphic DNA region adjacent to the 5' end of the human insulin gene. Proc. Natl. Acad. Sci. USA 78, 5759-5763.

Bodmer, J.G., Marsh, S.G.E., Albert, E.D., Bodmer, W.F., Dupont, B., Erlich, H.A., Mach, B., Mayr, W.R., Parham, P., Sasazuki, T. and Schreuder, G.M. (1991) Nomenclature for factors of the HLA system. Human Immunol. 34, 4-18.

Boehm, C.D., Antonarakis, S.E., Phillips, S.A., Steffen, G. and Kazazian, H.H. (1983) Prenatal diagnosis using DNA polymorphisms. Report on 95 pregnancies at risk for sickle-cell disease or /3-thalassemia. N. Engl. J. Med. 308, 1054.

Bugawan and Erlich, H.A. (1991) Rapid typing of HLA-DQBI polymorphism using nonradioactive oligonucleotide probes and amplified DNA. Immunogenetics 33, 163-170.

Cowchock, F.S., Smith, J.B., David, S., Scher, J., Batzer, F. and Corson, S. (1990) Paternal mononuclear cell immunization therapy for repeated miscarriage: predictive variables for pregnancy success. Am. J. Reprod. Immunol. 22, 12-17.

Erlich, H.A. and Bugawan, T.L. (1989) HLA class II gene polymorphism: DNA typing, evolution, and relationship to disease susceptibility. In: PCR Technology: Principles and Applications for DNA Amplification (Erlich, H.A., ed.), pp. 193-208. Stockton Press, New York.

Gill, T.J. ill and Repetti, C.F. (1979) Immunologic and genetic factors influencing reproduction. Am. J. Pathol. 95, 464-570.

Gyllenstein, V.B. and Erlich, H.A. (1988) Generation of single-stranded DNA by the polymerase chain reaction and its application to direct sequencing of the HLA-DQA locus. Proc. Natl. Acad. Sci. USA 85, 7652-7656.

Helmuth, R., Fildes, N., Blake, E., Luce, M.C., Chimera, J., Madej. R., Gorodezky, C., Stoneking, M., Schmill, N., Klitz, W., Higuchi, R. and Erlich, H.A. (1990) HLA-DQa allele and genotype frequencies in various human populations, determined by using enzymatic amplification and oligonucleotide probes. Am. J. Human Genet. 47, 515-523.

Higuchi, R. (1989) Simple and rapid preparation of samples for PCR. In: PCR Technology: Principles and Applications for DNA Amplification (Erlich, H.A., ed.). pp. 31-38. Stockton Press, New York.

Horn, G.T., Bugawan, T.L., Long, C. and Erlich, H.A. (1988) Allelic sequence variation of the HLA-DQ loci: relationship to serology and insulin-dependent diabetes susceptibility. Proc. Natl. Acad. Sci. USA 85, 6012-6016.

Komlos, L., Zamir, R., Joshua, H., Halbrecht, 1. (1979) Common HLA antigens in couples with repeated abortions. Clin. Immunol. Immunopathol. 7, 330-335.

Larson, A.E. (1983) A summary of the results of screening for HLA antibodies for one year. Transfusion 23, 268-269.

McIntyre, J.A. and Faulk, W.P. (1982) HLA and generation of diversity in human pregnancy. Placenta (Suppl.) 4, 1-1I.

McIntyre, J.A., Faulk, W.P., Nichols-Johnson, V.R. and Taylor, C.G. (1986) Immunologic testing and immunotherapy in recurrent spontaneous abortion. Obstet. Gynecol. 67, 169-174.

Nelson, J.L., Hughes, K.A., Smith, A.G., Nisperos, B.B., Branchaud. A.M. and Hansen, J.A. (1993) Maternal-fetal disparity in HLA class ll alloantigens and the pregnancy-induced ameliorations of rheumatoid arthritis. N. Engl. J. Med. 329, 466-471.

Ober, C. (1992) The maternal-fetal relationship in human pregnancy: An immunologic perspective. Exp. Clin. lmmunogenet. 9, 1-14. .

Ober, C., Lester, L.A., Billstrand, C., Mott, C., Lemke, A., van der Ven, K., Marcus, S., Kraut, J., Lloyd-Still, J. and Booth, C. (1992) Ethnic heterogeneity and CFTR mutation frequencies in Chicago area CF families. Am. J. Human Genet. 51, 1344-1348.

Ober, C., Elias, S., Kostyu, D.D. and Hauck, W.W. (1993) Prospective study of pregnancy outcome in Hutterites: decreased recognized fecundability in couples sharing HLA DR. Am. J. Human Genet. 50, 6-14.

Payne, R. and Rolfs, M.R. (1958) Fetomaternal leucocyte incompatibility. J. Clin. Invest. 37, 1756-1763.

Reznikoff-Etievant, M.F., Edelman, P., Muller, 1.Y., Pinon, F. and Sureau, C. (1984) HLA DR locus and maternal-fetal relation. Tissue Antigens 24, 30-34.

Saiki, R.K., Bugawan, T.L., Horn, G.T., Mullis, K.B. and Erlich, H.A. (1986) Analysis of enzymatically amplified beta-globin and HLA-DQa DNA with allele-specific oligonucleotide probes. Nature 324, 163-166.

Schacter, B., Muir, A., Gyves, M. and Tasin, M. (1979) HLA-A,B compatibility in parents of offspring with neural-tube defects or couples experiencing involuntary fetal wastage. Lancet i, 796-799.

Unander, A.M. and Olding, L.B. (1983) Habitual abortion: parental sharing of HLA antigens, absence of maternal-blocking antibody, and suppression of maternal lymphocytes. Am. J. Reprod. Immunol. Microbiol. 4, 171-178.

van der Ven, K., Kelly, M., Billstand, C., Steck, T. and Ober, C. (1992) Associations between MHC class II genes and unexplained infertility. J. Immunol.. Immunopharmacol. XII, 149. van

Rood, J.J., van Leeuwen, A. and Eernisse, J.G. (1958) Leucocyte antibodies in sera from pregnant women. Nature (London) 181, 1735-1736.