Immune parameters affecting maternal tolerance towards the fetus in normal and aberrant pregnancies

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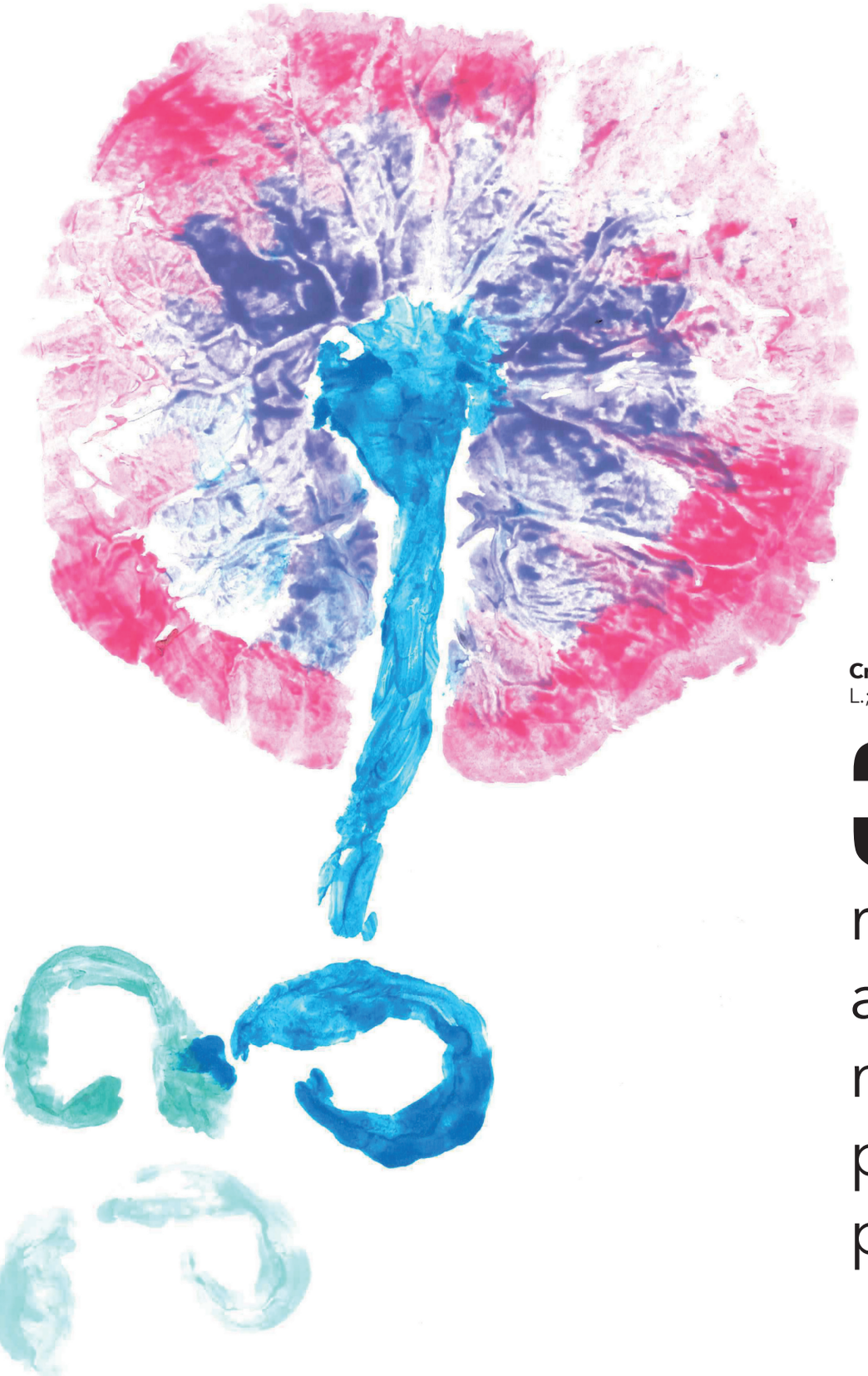

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reciprocal HLA-DR allogenicity between mother and child affects pregnancy outcome parameters

Abstract

Successful pregnancy outcome depends on local immunoregulatory mechanisms preventing a detrimental immune response towards the semi-allogeneic fetus. We investigated the influence of HLA-DR (in)compatibility on pregnancy outcome parameters in 480 women. The parameters tested were birth weight, individualized birthweight ratio (IBR), gestational age and maternal highest diastolic blood pressure. Irrespective of pregnancy complications, maternal-fetal HLA-DR incompatibility resulted in increased IBR. We conclude that reciprocal HLA-DR allogenicity between mother and child positively affect pregnancy outcome parameters.

Introduction

Successful pregnancy outcome depends on local immunoregulatory mechanisms preventing a detrimental maternal immune response towards the semi-allogeneic fetus. Paternally-inherited fetal HLA antigens can induce maternal immune activation and a variety of immune cells are recruited to the placental bed to secure and promote the pregnancy. Regulatory T cells (Tregs) play an important role in successful pregnancy. These Tregs are generally CD4+ and are thus HLA class II restricted. In organ transplantation, matching for HLA-DR leads to a better graft survival and function [1].

In the setting of pre-transplant blood transfusion it has been shown that at least one HLA-DR antigen has to be shared between donor and recipient in order to induce a tolerogenic effect on the course of a subsequent renal transplantation, while incompatibility for the second HLA-DR antigen enhances a stable, rejection-free, allograft function [2, 3].

In line with this blood transfusion concept, the pregnant mother has to accept the semi-allogeneic fetus. Trophoblast cells do not express HLA-DR, but fetal chimeric cells can cross the placenta and trigger a maternal immune response. Moreover, such transfer is bidirectional [4]. Both maternal and fetal cells can cross the placenta and fetal immune cells can also respond to maternal alloantigens.

Several studies have aimed at finding a correlation between pregnancy complications such as preeclampsia (PE) or recurrent miscarriage (RM) and the presence of certain HLA alleles, maternal homozygosity or sharing of HLA between mother and father or between mother and fetus. Recently, a systematic review showed that HLA-B sharing and HLA-DR sharing were both associated with the occurrence of recurrent miscarriage [5]. These results suggest that there is a negative correlation between HLA sharing and a favorable pregnancy outcome. This is in line with previous findings, suggesting that HLA sharing between mother

and child is associated with pregnancies complicated by PE [6]. These studies focused on pregnancy complications and do not necessarily represent the interaction of HLA molecules and immune cells during uncomplicated pregnancy. Therefore, we sought to take a different approach to examine the possible effect of HLA on pregnancy outcome with the use of objective parameters.

We conducted a retrospective, observational study to investigate the influence of fetal and maternal HLA-DR sharing on pregnancy outcome using objective outcome parameters as birth weight, gestational age and maternal highest diastolic blood pressure.

Materials & Methods

We retrospectively studied a cohort of 480 women who gave birth in the Leiden University Medical Center between 1992 and 2011, and their children. The majority of the pregnancies (59%) investigated were uncomplicated term pregnancies representing successful pregnancy. All women signed informed consent and the study was approved by the Ethics Committee of the Leiden University Medical Center. HLA-DRB1 typing of both mother and child was performed by SSO PCR technique using a reverse dot-blot method at the national reference laboratory for histocompatibility testing (Leiden University Medical Center, the Netherlands). We divided the woman-child pairs into four previously described groups [6] based on the degree of HLA-DR compatibility, as depicted in Figure 1. Maternal allogenicity was defined as the situation in which the mother expresses two distinct HLA-DR antigens and the fetus only expresses one allelic form. In the situation of fetal allogenicity the fetus expresses two distinct HLA-DR antigens, whereas the mother only expresses one allelic form. In the reciprocal allogenicity group both the mother and fetus express two distinct HLA-DR antigens of which one of the HLA-DR antigens is mismatched between mother and child. Syngenicity was

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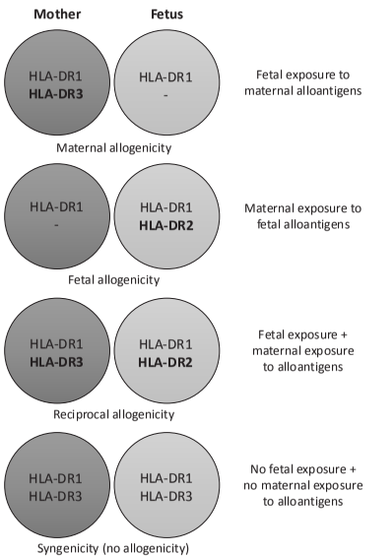

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Variable	Allogenicity	N	Median	Min	Max	P-value
IBR	Maternal	54	1.00	0.51	1.39	**0.030***
	Fetal	50	0.97	0.62	1.43	
	Reciprocal	309	1.06	0.44	1.49	
	Syngenicity	53	1.03	0.53	1.49	
Birth weight (gram)	Maternal	55	3100	657	4975	**0.029***
	Fetal	51	3170	879	4550	
	Reciprocal	319	3365	625	5285	
	Syngenicity	55	3230	990	4155	
Highest diastole (mmHg)	Maternal	52	80	65	120	0.939
	Fetal	50	80	60	110	
	Reciprocal	317	80	60	160	
	Syngenicity	53	80	60	130	
Gestational age (days)	Maternal	55	269	202	292	0.898
	Fetal	51	270	209	295	
	Reciprocal	319	271	190	297	
	Syngenicity	55	270	210	294	

* P <0.05. The p-values were calculated using the Kruskal-Wallis test.

Figure 1. HLA-DR allogenicity and pregnancy outcome. (A) The different types of maternal-fetal HLA relationships and potential for maternal and/or fetal exposure to alloantigens. Maternal allogenicity: the mother expresses two distinct HLA-DR antigens and the fetus only expresses one allelic form. Fetal allogenicity: the fetus expresses two distinct HLA-DR antigens, whereas the mother only expresses one allelic form. Reciprocal allogenicity: both the mother and fetus express two distinct HLA-DR antigens of which one of the HLA-DR antigens is mismatched between mother and child. Syngenicity: the mother and child express the same HLA-DR antigens. (B) Results of pregnancy outcome parameters for different HLA-DR allogenicity groups.

defined as the situation in which the mother and child express the same HLA-DR antigens.

The parameters tested were birth weight, individualized birthweight ratio (IBR), gestational age and maternal highest diastolic blood pressure. The IBR is a ratio of the actual birthweight divided by the predicted birthweight [7]. It is calculated by dividing the actual birth weight by the mean birth weight of children of the same sex born after a pregnancy with equal parity and gestational age, as derived from the Kloosterman tables [8]. Supplementary Tables S1 and S2 show the characteristics of the study population.

All other statistical analyses were performed using SPSS Statistics 23 software (IBM SPSS Software, New York, USA). Non-parametric tests were used, since data were not normally distributed according to the Shapiro-Wilk normality test. The Kruskal-Wallis test was used to analyze the distribution of the pregnancy outcome parameters between the different HLA-DR groups. P-values lower than 0.05 were considered statistically significant. To test for independent effects of HLA-DR on pregnancy outcome parameters, we included covariates in a regression model. Inclusion criterion for inclusion in the multivariate analysis was a univariate P-value of <0.1.

Results and discussion

The present study showed that reciprocal allogenicity is significantly related to a higher IBR (Figure 1). The group in which both the mother and fetus express two distinct HLA-DR antigens, with one HLA-DR mismatch between mother and child, had the highest birth weight (P=0.029) and IBR (P=0.030). After correction for maternal age, gravidity, parity, spontaneous abortion, PE/HELLP and smoking, we found a trend for reciprocal HLA-DR allogenicity and birth weight (P=0.068). The association between reciprocal HLA-DR allogenicity and IBR was independent of these factors (P=0.042). The IBR is a superior measure for abnormal and normal growth, because this factor effectively controls for physiological birthweight determinants. These results indicate that the optimal situation for pregnancy is reciprocal allogenicity. Our results suggest that incompatibility for one HLA-DR antigen between mother and fetus leads to triggering and activation of the immune response, while the other HLA-DR antigen has to be shared in order to induce immune regulation. Since reciprocal allogenicity was the most optimal situation found in our study, both fetal and maternal immune responses seem to be important. Although trophoblast cells do not express HLA-DR, HLA-DR+ fetal chimeric cells can cross the placenta [4] and interact with the maternal immune system leading to a similar immune regulation as previously has been described for pretransplant blood transfusions [2]. During pregnancy, increased numbers of CD4+ Tregs are indeed present in the decidua and contribute to the regulation of fetus-specific responses [9].

Similarly, HLA-DR+ chimeric maternal cells in the fetus will interact with the developing fetal immune system, leading to the establishment of a large pool of fetal Tregs [10]. This T cell tolerance towards maternal alloantigens perceived in utero may even be maintained after birth through the establishment of long-lived Tregs, which play a crucial role in the clinical observations showing that mismatches for non-inherited maternal antigens (NIMAs) are better tolerated than non-inherited paternal alloantigens in the setting of adult solid organ transplantation [11].

The percentage of preterm births in this study (26%) is quite high. This is the direct result of collecting retrospective data from women who gave birth in a Dutch academic hospital. In the Netherlands it is still common to give birth at home under supervision of a midwife, which will have led to a relatively high percentage of deliveries with pregnancy complications in hospitals.

We did not collect any information on socioeconomic status, marital status, education, and race-ethnicity. Even though we think it is unlikely that these variables would have influenced the effect of HLA-DR allogenicity on pregnancy outcome parameters, we cannot fully exclude the effect of these factors.

In summary, we conclude that the most optimal situation for a successful pregnancy is that of reciprocal HLA-DR allogenicity. This suggests that active induction of immune tolerance from both maternal and fetal side is important.

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Supplementary Table S1. Maternal characteristics of the 480 women included in the study.

Mother		n
Age (years) *	33 (19-46)	480
Highest diastolic pressure (mmHg) *	81 (60-160)	472
Proteinuria (positive) #	56 (11.7%)	480
Gravidity*	3 (1-10)	480
Parity*	1 (0-6)	480
Previous spontaneous abortions*	1 (0-7)	480
Smoking#		480
- No smoking	409 (85.2%)	
- 1-10 cigarettes/day	18 (3.8%)	
- >10 cigarettes/day	10 (2.1%)	
- Unknown	43 (9.0%)	

* Mean value with the range between parentheses. # Number with the percentage of the total population.

Supplementary Table S2. Pregnancy characteristics of the 480 women included in the study.

Pregnancy		n
Gestational age (days) *	264 (190-297)	480
Mode of delivery#		480
- Spontaneous	200 (41.7%)	
- Caesarean section	280 (58.3%)	
Indication primary caesarean section (n= 246) #		246
- Breech presentation	70 (28.5%)	
- Caesarean previous pregnancy	59 (24.0%)	
- Obstetric medical history	23 (9.3%)	
- Maternal/Fetal indication	16 (6.5%)	
- Other	78 (31.7%)	
Indication secondary caesarean section (n= 34) #		34
- Failure 1st stage	5 (14.7%)	
- Failure 2nd stage	7 (20.6%)	
- Maternal indication	2 (5.9%)	
- Fetal indication	15 (44.1%)	
- Other	5 (14.7%)	
Child		
Birth weight (gram) *	3090 (625-5285)	480
Gender (male) #	236 (49.2%)	480
Placenta weight (gram) *	559 (100-1480)	381
Complications		
Pre-eclampsia#	47 (9.8%)	480
HELLP#	7 (1.5%)	480
IUGR (<5th percentile)#	22 (4.6%)	480
Preterm (<37 weeks)#	123 (26%)	480

* Mean value with the range between parentheses. # Number with the percentage of the total population.

Supplementary Table S3. Factors affecting the effect of HLA-DR reciprocal allogenicity on birth weight and IBR.

		Univariate regression				**Multivariate regression**			
			95% C.I.				**95% C.I.**		
		β	**Lower**	**Upper**	**P-value**	**β**	**Lower**	**Upper**	**P-value**
Birth weight (gram)	Maternal age	28.394	11.47	45.32	0.001*	19.671	4.97	34.38	0.009*
	Gravidity	88.767	36.47	141.07	0.001*	-23.598	-84.31	37.12	0.445
	Parity	168.622	89.87	247.38	0.000*	58.956	-32.97	150.89	0.208
	Spontaneous abortions	35.481	-45.59	119.56	0.407				
	PE/HELLP	-1799.130	-2018.50	-1579.76	0.000*	-1698.406	-1928.28	-1468.53	<0.001*
	Smoking	-419.493	-756.11	-82.87	0.015*	-264.964	-536.73	6.80	0.056
	DR reciprocal allogenicity	165.293	-10.40	340.99	0.065	130.951	-9.95	271.85	0.068
IBR	Maternal age	0.002	0.00	0.01	0.154				
	Gravidity	0.013	0.00	0.02	0.016	0.000	-0.01	0.01	0.974
	Parity	0.025	0.01	0.04	0.002*	0.011	-0.01	0.03	0.284
	Spontaneous abortions	0.004	-0.01	0.02	0.622				
	PE/HELLP	-0.217	-0.27	-0.17	0.000*	-0.205	-0.26	-0.15	<0.001*
	Smoking	-0.094	-0.16	-0.03	0.005*	-0.077	-0.14	-0.02	0.013*
	DR reciprocal allogenicity	0.045	0.01	0.08	0.010*	0.033	0.00	0.07	0.042*

β = regression coefficient. C.I. = Confidence interval. * P <0.05. The p-values were calculated using linear regression.