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Prevalence and qualitative properties of circulating anti–human leukocyte antigen alloantibodies after pregnancy: No association with unexplained recurrent miscarriage

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ABSTRACT

In pregnant women, circulating alloantibodies, triggered by exposure to paternal HLA antigens, are frequently detectable. The finding of lower alloantibody levels in women who experience spontaneous abortion (miscarriage) has led to the speculation that antipaternal antibodies could favor maintenance of pregnancy, whereas their lack poses a risk of miscarriage. Postulating a role of alloantibodies in the pathogenesis of unexplained abortion, we examined whether different categories of recurrent miscarriage (RM) can be distinguished according to prevalence or distinct qualitative properties of anti–human leukocyte antigen (HLA) antibody patterns. Sera obtained from 167 women with RM were assessed for complement- and non–complement-fixing anti-HLA alloreactivity using Luminex-based bead array technology. Women with RM had less often detectable anti-HLA class I and/or II reactivity (19%) compared with a control group of 96 multiparous women without a history of miscarriage (49%). However, analysis of different categories of RM (unknown [$n = 112$] versus known cause [$n = 55$]; primary [$n = 125$] versus secondary RM [$n = 42$]) did not reveal any differences regarding antibody prevalence, number of targeted HLA single antigens, antigen specificity, binding density, or complement-fixing ability of detected alloantibodies. Our results do not support a link between anti-HLA antibody formation and RM, and argue against a diagnostic value of alloantibody detection in the diagnostic work-up of women with RM.

1. Introduction

In the late 1950s, it was recognized that pregnancy triggers an alloimmune response against paternally derived fetal alloantigens that frequently results in the formation of circulating alloantibodies [1,2]. The actual physiologic relevance of these antipaternal antibodies (APA) in the maintenance of pregnancy, however, is unclear. In light of its high prevalence in normal pregnancy, alloantibody formation is commonly assumed to represent a harmless phenomenon. Indeed, earlier studies did not provide evidence for an association between alloantibody formation and miscarriage or obstetric complications [3,4]. Of note, previous studies have shown that APA detected by leukoagglutination or complement-dependent cytotoxicity (CDC) are less prevalent among women with a spontaneous miscarriage, and some authors have concluded that the failure to mount a significant maternal alloimmune response could be related to pregnancy loss [5–8]. The early assumption of a protective role of humoral alloimmunity has prompted intervention studies in women with RM designed to lower the risk of future miscarriage by active immunization with paternal or third-party lymphocytes. However, the results of randomized trials to assess the efficiency of immunotherapy have been conflicting [9–11]. An alternative explanation for reduced anti-HLA reactivity in women who experience miscarriage or spontaneous abortion (aborters) could be the comparably short exposure to paternal alloantigens. In fact, seroconversion was reported to peak not until the second trimester [12,13], and a large prospective study of sequential antibody detection did not demonstrate any differences between successful and failed pregnancy at early stages [13]. Finally, some authors have suggested deleterious effects of anti-HLA reactivity, and a recent study, performed in women with unexplained RM, has suggested a link between anti-HLA antibody formation in early pregnancy and a reduced chance of live birth [14].

In many cases, the etiology of RM remains unclear (idiopathic RM), even after thorough evaluation, and a variety of candidate causes, including immunologic factors, were hypothesized to contribute to RM [11]. Regarding the suggested role of anti-HLA anti-

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bodies in RM, one may argue that idiopathic RM differs from RM of known etiology (where an alloimmune cause may be less probable) in terms of prevalence or distinct qualitative properties of alloantibody patterns, such as the ability to activate the classical complement pathway. In earlier studies, alloantibodies were reported to be less frequent in women who had lost all previous pregnancies (primary abortion) compared with those who experienced secondary abortion [15,16]. Recently, an increased prevalence of anti-HLA reactivity was noted among secondary aborters with a firstborn male infant, which was suggested to be related to an increased fetal cell microchimerism [14]. Hitherto however, no study has addressed the question whether there is a different anti-HLA antibody pattern in women with unexplained RM compared with RM of known etiology.

The objective of the present study was to clarify whether distinct forms of RM, such as idiopathic RM and primary RM, are associated with a particular anti-HLA antibody pattern that distinguishes them from other types of RM. For detailed serologic analysis, we used cell-independent bead array technology for solid-phase detection of HLA single antigen (SA) reactivities. Sensitive and specific anti-HLA antibody detection enabled us to analyze a variety of specific properties of detected antibodies, including binding intensity, antigen specificity, and the ability to activate the classical complement pathway.

2. Subjects and methods

2.1. Subjects

In this retrospective study, 263 sera (sampling between 2000 and 2008 at the Medical University of Vienna) obtained from 167 women with RM (two miscarriages, $n = 24$; three miscarriages, $n = 86$; more than three miscarriages, $n = 57$) and 96 multiparous women without a history of miscarriage as controls were subjected to anti-HLA antibody detection. As shown in Table 1, most sera were obtained within the first year after the last delivery (abortion or successful pregnancy, respectively). Before testing samples were stored at −80°C. The study was approved by the local ethics committee (ethical approval no. 703/2007) and sera were obtained after written informed consent.

Miscarriage was defined as pregnancy that fails to progress, resulting in the death and expulsion of the embryo or fetus, stipulating that the fetus or embryo should weight ≤500 g, a stage that corresponds to a gestational age of less than 20–22 weeks [17]. In our cohort, RM was defined as two or more successive miscarriages. Multiparous women were enrolled in the study if they had two or more successful pregnancies and no history of miscarriages. As shown in Table 1, women with RM were slightly older than multiparous controls (34 versus 33 years, $p = 0.02$), had a higher number of immunizing events (three miscarriages and/or successful pregnancies versus two successful pregnancies, respectively, $p < 0.0001$) and the interval between the last immunizing event and serologic analysis was shorter (8 versus 4 months, $p = 0.02$).

All women with RM were screened for genetic aberrations (maternal and paternal karyotyping), uterine anatomic abnormalities, antiphospholipid syndrome, coagulation abnormalities, systemic viral infections, including hepatitis C, genital tract infections, and endocrine abnormalities (*e.g.*, polycystic ovary syndrome, hypothyroidism). In 112 women with RM, a complete assessment did reveal any cause of miscarriage, and these cases were classified as idiopathic RM. For 55, women a specific cause of abortion could be identified (RM of known origin; anatomic cause, $n = 38$; antiphospholipid syndrome, $n = 3$; chromosomal aberration, $n = 2$; coagulation abormalites, $n = 11$; polycystic ovary syndrome, $n = 1$). A total of 125 women who lost all previous pregnancies were classified as primary RM, and 42 women who had one or more successful pregnancies followed by consecutive miscarriages were categorized as secondary RM [17]. The proportion of secondary RM was similar among women with idiopathic RM versus RM of known cause (24% versus 27%, respectively). Comparing idiopathic RM with RM of known origin, or primary with secondary RM, respectively, we found no statistically significant differences in the number of immunizing events (successful and/or failed pregnancy) or timing of serum collection (Table 1). However, there was a trend toward an older age in secondary compared with primary aborters.

2.2. HLA antibody detection

In a first step, sera were prescreened applying FlowPRA HLA class I and II screening (One Lambda, Inc., Canoga Park, CA). In brief, HLA class I or class II beads were incubated for 30 minutes with undiluted serum and after washing, incubated with allophycocyanin-conjugated polyclonal donkey anti-human IgG antibody (Jackson Immuno Research Europe, Soham, UK), at saturating concentration for another 30 minutes. Fluorescence intensity was measured using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA). Markers were set according to an appropriate nonbinding negative control serum obtained from a healthy male volunteer. Results were expressed as percentage of positive events (percentage of panel-reactive antibodies [PRA]). A serum was considered antibody positive if PRA levels were equal to or greater than 10%.

In a second step, FlowPRA HLA class I–positive and/or II–positive sera were evaluated for anti-HLA SA reactivities using Luminex-based SA analysis for HLA class I or II ([IgG] Luminex; HLA class I: LABScreen Single Antigen HLA Class I Antibody Detection Test–Combi; HLA class II: LABScreen Single Antigen HLA Class II Antibody Detection Test–Group 1; One Lambda, Canoga Park, CA), according to the manufacturer's protocol. A phycoerythrin-conjugated goat antihuman IgG antibody (One Lambda) was used for anti-HLA antibody detection.

Table 1
Demographic results according to success of pregnancy and types of recurrent miscarriage

Parameter	Multipara	RM	*P* value	Classification of RM according to:					
				Identification of a specific cause		*P* value	Previous successful pregnancy		*P* value
				Idiopathic RM	RM of known cause		Primary RM	Secondary RM	
Subjects, *n*	96	167		112	55		125	42	
Age median, y (IQR)	33 (29–35)	34 (30–39)	0.02	33 (29–39)	35 (31–38)	0.6	33 (29–38)	35 (32–39)	0.07
Miscarriages/live births, median (IQR)	2 (2–3)	3 (3–4)	<0.0001	3 (3–4)	3 (3–4)	0.9	3 (3–4)	4 (3–5)	0.1
Last event to sampling, median, mo (IQR)	8 (4–13)	4 (2–12)	0.02	4 (2–13)	4 (2–12)	0.7	4 (2–9)	5 (2–24)	0.3

IQR, interquartile range; RM, recurrent miscarriage.