Immunology of Recurrent Spontaneous Abortion

A Review

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Traditionally, four main causes are considered in evaluating patients with a history of recurrent spontaneous abortion: infection, chromosome abnormalities, low progesterone levels, and anatomic abnormalities. However, the majority of such losses are mediated by immune dysfunction, including inadequate maternal antipaternal leukocyte antibodies and the presence of antiphosphlipid antibodies and antinuclear antibodies. Immunologic problems are easily identified and treated using modalities such as paternal leukocyte immunization, prophylactic heparin, baby aspirin, and low-dose prednisone. With appropriate and timely treatment, the success rate is approximately 80%.

Introduction

The immunology of reproduction is a dynamic field, with data forthcoming exponentially. Immune mechanisms are operative in infertility, endometriosis, eclampsia/preeclampsia, miscarriage, and other aspects of reproduction. This paper focuses on the immunology of recurrent spontaneous abortion (RSA). Classically, a patient is considered to have RSA if she has had three or more consecutive miscarriages, but many clinicians are now evaluating couples after two consecutive losses.

The causes of RSA have been classified as infection (1%), anatomic abnormalities (5% to 10%), lutealphase defect (5% to 20%), chromosomal abnormalities (7% to 50%), immune mechanisms (50%), and unknown (15%). Some women have multiple reasons for RSA. A workup comprising ultrasonography, hysterosalpingography, laparoscopy, endometrial biopsy, parental and fetal chromosome analysis, cervical culture, and progesterone testing would explain only about 50% of the pregnancy losses. There is strong evidence that the remainder of miscarriages are mediated by immune mechanisms.[1-3]

The uterus is an enigma. Despite a full complement of immunocompetent cells, it allows the fetal allograft to thrive for 40 weeks. During pregnancy, the fetoplacental unit orchestrates immune mechanisms via T and B lymphocytes, natural killer cells (NK), a variety of soluble immunoregulatory factors (cytokines), and antibodies. To a significant degree, the interaction between maternal and fetoplacental tissue and the immune system will determine whether a pregnancy succeeds. Three antibodies are critically important to pregnancy maintenance: maternal antipaternal leukocyte antibodies (APLA) (ie, blocking antibodies), antiphospholipid antibodies (APA), and antinuclear antibodies (ANA).

When the immune system is the cause of miscarriage, the mother has a 30% chance of having a successful pregnancy without intervention after 3 miscarriages, a 25% chance after 4 miscarriages, and a 5% chance after 5 miscarriages. With proper treatment, the overall success rate has been reported at 70% to 85% in parity and age-matched controls.[1-3]

Antipaternal Leukocyte Antibodies

APLA are antibodies that mask paternal human leukocyte antigens (HLA) found on the fetus from maternal immune effector cells. Genes that code for HLA or tissue type are located on chromosome 6.[4] HLA consist of class I and class II antigens. Class I antigens, which include the A, B, and C loci, are found on all nucleated cells and platelets and are the only HLA expressed on nonactivated T lymphocytes. More recently, another class I HLA, designated G, has been identified on cytotrophoblasts versus syncytiotrophoblasts, which do not express any HLA.[5] However, studies using the polymerase chain reaction have shown that the placental barrier is not impervious to tissue, so that maternal cells have been found in the fetal circulation and fetal cells in maternal circulation.[6]

A more limited number of cells (eg, B lymphocytes, macrophages, monocytes, dendrites, activated T lymphocytes) express class II antigens, which comprise the DR, DQ, and DP loci. There is sufficient HLA polymorphism that immuno-identity is unique to each individual (Table 1). B cells are the immune response cells capable of producing antibodies. When a woman becomes pregnant, uterine lymphocytes produce APLA against the father's HLA. APLA have been demonstrated as early as 5 weeks' gestation, and protect the fetus from maternal NK cells that are capable of rejecting the fetus.[7]

An early observation in couples who suffer RSA was the degree of HLA congruity—most commonly at the B,

Table 1. HLA Phenotypes

A locus		B locus		C locus	DR locus	DQ locus
A1	A33(19)	B51 (5)	B57(17)	Cw1	DR1	DQ2
A36	A11	B52(5)	B58(17)	Cw2	DR4	DQ4
A2	A68(28)	B53	B18	Cw3	DR7	DQ5(Q1)
A3	A69(28)	B7801	B49(21)	Cw4	DR8	DQ6(Q1)
A23(9)	A29(19)	B7	B50(21)	Cw5	DR9	DQ7(Q3)
A24(9)	A30(19)	B42	B54(22)	Cw6	DR10	DQ8(Q3)
A25(10)	A31(19)	B67	B55(22)	Cw7	DR11 (5)	DQ9(Q3)
A26(10)	A32(19)	B8	B56(22)	Cw8	DR12(5)	
A34(10)	A74(19)	B59	B27		DR13(6)	
A66(10)		B44(12)	B47		DR 14(6)	
		B45(12)	B35		DR15(2)	
		B13	B37		DR 16(2)	
		B64(14)	B60(40)		DR 17(3)	
		B65(14)	B61 (40)		DR18(3)	
		B62(15)	B48			
		B63(15)	B41			
		B75(15)	B71 (70)			
		B77(15)	B72 (70)			
		B38(16)	B73			
		B39(16)	B46			

DR, and DQ loci.[8,9] It has been postulated that this lack of disparity interferes with the production of alloantibodies. Multiparous women generally have circulating APLA even in the nonpregnant state, whereas habitual aborters have undetectable or low levels (Table 2).

In the RSA couple, APLA levels should be ascertained prior to conception using cell-flow cytometry.[10] The husband's Iymphocytes are combined with the wife's serum (which would contain APLA if present), and incubated with fluorescent markers. The entire mixture is placed into the cytometer, which utilizes laminar flow fluidics and argon lasers. Under laser illumination, cells that have APLA attached will fluoresce. The emission will be captured by photomultiplier tubes and transferred to a computer that digitizes the signal.

FAST TAKE

Treatment of patients with APA is more effective when medication, if indicated, is started **prior to conception** and continued throughout pregnancy.

The presence of APLA can also be confirmed indirectly via microcytotoxicity and mixed Iymphocyte reaction (MLR). The drawback to these testing modalities is that they depend on numerous variables; for example, the former measures only antibodies that fix complement, and MLR depends on culture conditions. To date, no research has validated these tools in predicting pregnancy outcome.

Treatment involves immunizing the mother with concentrates of paternal Iymphocytes so that the signal is amplified approximately 10,000 times the level normally seen in early pregnancy.[1] Paternal leukocyte immunization (PLI) treatments are usually administered 4 weeks apart. Four weeks after the second immunization, the APLA level is remeasured. When APLA is appropriately elevated prior to conception, the rate of successful term pregnancy is approximately 80% (Table 3). The efficacy of various human gamma globulin monomers is currently under investigation.[11]

PLI carries the risk of possible transmission of infectious agents such as hepatitis A, B, and C; human immunodeficiency virus (HIV); and human T-cell leukemia virus I (HTLV-l). Paternal blood is routinely tested for these viruses before use. In rare cases, cellulitis can develop. PLI facilitates the production of certain alloantibodies but not autoantibodies, so that induction of autoimmune disease is undocumented.[12]

Antiphospholipid Antibodies

Phospholipid molecules are normal components of all cell membranes. Antibodies to phospholipids have been implicated in numerous disease states, generating much academic interest. APA are capable of vascular compromise via damage to vascular endothelium and platelet

Table 2. Comparison of APLA Levels in RSA Patients and Nonaborters

Group	Blocking Ab Present Prior to Pregnancy	Delivered
Women with no miscarriages	Yes (82/100)	100/100(100%)
Women with miscarriages (RSA)	No (6/175)	0/175 (0%)

Ab = antibody
RSA = recurrent spontaneous abortion
Kiprov, et al; J Immunol Immunopharmacol.1992;12:108.

membrane by inhibiting prostacyclin (vasodilator) and interfering with the activation of protein C.[13] The result is increased platelet adhesion and a relative rise in thromboxane (vasoconstrictor), resulting in a milieu conducive to thrombotic events. In the uteroplacental circulation these insults translate into fetal demise or intrauterine growth retardation.

FAST TAKE
Patients with APA can be treated by using baby aspirin and heparin throughout pregnancy, and should be evaluated for the presence of underlying autoimmune conditions.

Some phospholipid molecules—particularly phosphoserine and phosphoethanolamine—have adhesive properties. They allow cells to fuse so that in the placenta, cytotrophoblasts become syncytiotrophoblasts, which regulate nutrients to the fetus. A study on the formation of syncytia in the trophoblastic cell line Be-Wo discovered that monoclonal anti bodies to phosphoserine (but not to cardiolipin) inhibited the formation of syncytia in vitro.[14] Furthermore, placentas from patients with APA who miscarried had a high percentage of immunoglobulin M (IgM) APA bound to the syncytia. Interference with cell adhesion by APA may predate clotting abnormalities.

FAST TAKE
The presence of ANA, which is common in SLE patients and can cause inflammatory processes in the uterus and placenta, can be countered by using prednisone.

With each pregnancy loss, there is a 10% chance that the mother will develop an antibody to a phospholipid molecule, and the effect is cumulative.[15] Most women with APA are asymptomatic, but some have underlying autoimmune tendencies and should be evaluated appropriately. Although there is a high incidence of APA in patients with systemic lupus erythematosus (SLE), there is a significant population who have APA but no other disease. The diagnosis assigned to patients with thrombotic events in the presence of APA is primary antiphospholipid antibody syndrome.

Treatment of APA involves the use of low-dose (baby) aspirin and prophylactic heparin,[16] which is a large molecule that cannot cross the placenta. Heparin activates the

Table 3. Success Rates in RSA Patients Treated for Alloimmune and Autoimmune Abnormalities

Group	Received PLI	APA and/or ANA	Medications Given	Delivered Pt. No. (%)
I (no treatment)	No	No	No	14/60 (23)
II	Yes	No	No	05/126 (83)
III	Yes	Yes	No	6/28 (21)
IV	Yes	Yes	Yes (early)	43/54 (80)
V	Yes	Yes	Yes (late)	8/21 (38)

Medication tor APAs and ANAs included low-dose aspinn, heparin, and prednisone, when indicated.
ANA = antinuclear antibodies
APA = antiphospholipid antibodies
PLI = paternal leukocyte immunization
Kwak JYH, Gilman-Sachs A, Beaman KD, Beer AE. Am J Obstet Gynecol 1992;166:1787-1795.

formation of antithrombin III, which interferes with the coagulation cascade. Although aspirin can traverse the placenta, the dose is small and usually does not affect the fetus. Aspirin inhibits cyclooxygenase and the formation of thromboxanes, allowing prostacyclin to act unopposed. Treatment is more effective when medication, if indicated, is started prior to conception and continued throughout pregnancy.

Antinuclear Antibodies

There is an increased prevalence of RSA patients who demonstrate ANA compared with parity- and age-matched nonaborters. What causes these antibodies to be synthesized is currently under investigation, but there appears to be a genetic susceptibility dictated by the HLA tissue type. This is compounded by the production of autoantibodies like ANA with fetal demise. The disease typically associated with ANA is SLE, which confers a much higher miscarriage rate than that of the general population— approaching 50% in patients with active disease.[17] Although most women with RSA do not fulfill the American College of Rheumatology criteria for SLE, many exhibit lupus-like tendencies. Polyclonal B cell activation appears to be more common in these patients.[18] Although the exact mechanisms whereby ANA contribute to miscarriage is unknown, placental pathology studies reveal inflammatory changes in the uterine and placental tissue (villitis) and vasculitis.

When ANA are present in the context of RSA, prednisone is recommended to suppress the inflammatory process and stabilize cell membranes.[19] Prednisone does not cross the placenta easily because it is highly bound to albumin, which is a large protein molecule. In addition, the placenta contains Beta2-dehydrogenase, which metabolizes this steroid. Suppression of the fetal adrenal axis has not been reported. When indicated, prednisone is instituted prior to conception. With treatment, there is an 80% to 85% chance of successful term pregnancy. As the body is dynamic, antibody levels may change over time. Patients who develop new autoantibodies during pregnancy have a more guarded prognosis.

Conclusion

Failure of maternal response to the fetal allograft, as well as the production of autoantibodies, can result in repetitive pregnancy loss. Contrary to popular belief, miscarriage is not a benign process, as the patient may develop autoantibodies. Fortunately, these problems are easily identified and amenable to treatment. Miscarriage due to immune dysfunction is largely preventable today, and couples desiring parenthood should be given appropriate consideration and evaluation. TFP

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