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Anticardiolipin Antibodies in Women with Unexplained Infertility

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Summary

Concept of autoimmune basis of infertility is still controversial, particularly regarding the presence of non-organ specific autoantibodies. Non-organ specific anticardiolipin (aCL) and antithyroglobulin (TgAt) antibodies were detected in infertile women. Both partners were evaluated according to the criteria of The American Society for Reproductive Medicine. All the results were normal in cases of unexplained infertility. Antisperm antibodies (ASA) were determined by a mixed antiglobulin reaction (MAR) and the Kibrick agglutination assay, aCL by commercial ELISA, TgAt by commercial RIA. Fertile women had children. Subjects were grouped in fertile (n=27), infertile (n=65), and cases of unexplained infertility (n=42). In fertile women, aCL was below the negative cut-off value (100 %), while women with unexplained infertility were positive in 23.8 %. Anticardiolipin (aCL) antibodies were detected in 21.5 % of infertile patients, most of them with unexplained infertility (15.4 %). Other positive women had partners with ASA (4.6 %), or exhibited a negative postcoital test (1.5 %). In this study aCL were not detected in women with ASA. TgAt incidence was increased in infertile (20 %) and unexplained infertility group (21.4 %) compared to the fertile controls (18.5 %). Increased incidence of aCL and TgAt in infertile women supports the contention that these autoantibodies contribute to the infertility.

Key words

Infertility • Anticardiolipin antibodies • Antithyroglobulin antibodies

Introduction

The investigations of causes of infertility and fetal loss are beginning to deal increasingly with immunological factors, and in particular, with the autoimmunity. Autoimmune processes and mechanisms are considered as contributing factors in some cases of unexplained infertility in both men and women, particularly when they are directed to antigens expressed in tissues and cells of the reproductive tract and genital secretion. Presence of antibodies to testicular antigens and sperm in men, or antibodies to zona pellucida and endometrial antigens in women, are considered possible causes of infertility in a fraction of patients with unexplained infertility. Presence of these organ-specific autoantibodies (Coulam and Stern 1992, van Voorhis and Stovall 1997) was important for acceptance of autoimmunity as a possible cause of infertility. However, the presence of other autoantibodies, which are not particularly organ-specific, such as antiphospholipid,

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antismooth muscle, antithyroid, or antinuclear antibodies, have recently been increasingly implicated (van Voorhis and Stovall 1997, Cubillos *et al.* 1997, Fialová *et al.* 2002a, 2002b). Some investigations showed a greater incidence of these antibodies compared to organ-specific antibodies in infertile women and the association between their presence and the period of infertility (Gleicher *et al.* 1993).

Antiphospholipid antibodies (aPL) are acquired autoantibodies directed to phospholipids which are associated with slow progressive thrombosis and infarction of the placenta (Branch *et al.* 1997). Clinical manifestations (venous or arterial thrombosis and recurrent spontaneous abortions) accompanied with the respective laboratory findings qualify for diagnosis of the antiphospholipid syndrome (APS). Antiphospholipid antibodies represent a family of antibodies of different specificities, most of which are directed towards different anionic phospholipids, which include cardiolipin, phosphatidylcholine, phosphatidylserine, phosphatidic acid, and phosphatidyl ethanolamine (Kutteh *et al.* 1999). Anticardiolipin antibodies react with a plasma cofactor β-2 glycoprotein 1 (β-2 GPI) that stabilizes antigenic conformation of cardiolipin, so that anticardiolipin antibodies represent a mixture of antibodies to β-2 GPI and antibodies to phospholipid epitopes stabilized by interaction with β-2 GPI (Chamley 1997, Kutteh *et al.* 1999). Reproductive failure that is associated with anti-phospholipid antibodies includes spontaneous abortion in the second or third trimester and pregnancy-induced hypertension. Gleicher and El-Roeiy (1988) introduced a concept of the autoimmune reproductive failure syndrome to stress the autoimmune cause of these defects. The low incidence of chromosomal abnormalities in these abortions supports pathogenetic significance of aPL (Takakuwa *et al.* 1997). Formation of aPL antibodies seems to be related to human leukocyte antigens (HLA) of the major histocompatibility complex, since some of the HLA alleles are associated with aPL antibodies in recurrent spontaneous abortions (Hataya *et al.* 1998).

Mechanisms of aPL-induced reproductive failure are not sufficiently well understood, but there is feto-placental insufficiency, hypoxia and intrauterine fetal death (Kutteh at al. 1999, Arakawa *et al.* 1999). In patients with recurrent spontaneous abortions, most frequent aPL antibodies are aCL (Yetman and Kutteh 1996), and their determination is considered the most sensitive test for aPL antibodies (Khamashita and Hughes 1996). Investigations of aCL-induced abortions in experimental animals have shown the direct effect of aCL on miscarriages (Bakimer *et al.* 1992). Cases of infertility in the course of the experiment have led the authors to consider aCL involvement in unexplained infertility (Sherer and Shoenfeld 1998). The experimental data on animal models and sporadic data on elevated levels of aPL antibodies in women with unexplained infertility (Roussev *et al.* 1996, Balasch *et al.* 1996) yielded a hypothesis of pathogenetic involvement of aPL in unexplained infertility (Sherer and Shoenfeld 1998). However, the occurrence of aPL and/or other organ non-specific antibodies can rather be considered as an epiphenomenon characteristic of the generalized activation of the immune system, common in autoimmune diseases rather than the direct cause of infertility (Gleicher 1998).

This study investigated aCL antibodies in fertile and infertile women. The aCL and Tg antibodies were determined in patients with unexplained infertility, showing higher occurrence in the later group.

Methods

Our investigation included infertile (n=65, mean age 34±6 years) and fertile couples (n=27, mean age 28±7 years). In infertile patients, a standard procedure of infertility evaluation according to *The Practice Committee Report of the American Society for Reproductive Medicine* (1992) was employed. This procedure included sperm analysis, determination of ovulation, postcoital test, hysterosalpingogram and, if indicated, laparoscopy. All patients were negative for Chlamydia, Mycoplasma and Ureaplasma, and no clinically recognized source of infertility was found. Hysterosalpingographic and laparoscopic findings were normal, as well as hormone profiles (judged by levels of LH, FSH, etradiol and progesterone). Time of ovulation was determined by basal temperature and ultrasonic folliculometry. In all male partners normal urological findings were present (spermograms and sperm cultures).

Postcoital test was performed in all women between days 11 and 14 of the menstrual cycle. Vaginal smear and cervical aspirates were taken 3-5 h postcoitum, and both presence and mobility of sperm was analyzed. The test was considered negative when only immotile sperm cells were found, weakly positive if only

non-progressively motile, and positive if progressively motile sperm cells were found in cervical aspirates.

Women of the fertile group were selected to have two children, last childbirth 1-2 years previously, and normal bacteriological results. At the time the samples were taken, no women were under therapy or using contraception.

For antibody tests, blood samples were taken between days 11 and 14 of the menstrual cycle (from the first day of the last menstrual period). Anticardiolipin antibodies were analyzed in sera using a commercial ELISA test with cardiolipin alone as antigen (Shield-Axis Diagnostics Ltd, Dundee, UK). Anticardiolipin antibody concentrations were determined using a standard curve based on a series with known concentrations of antibodies and expressed in GPL Units (aCL antibodies of IgG class) and MPL Units (aCL antibodies of IgM class). Negative cut-off values (10 GLP or 10 MLP) were based on the mean ± 3 S.D. of 110 subjects of both sexes. Antithyroid antibodies were determined using radioimmunoassay (RIA, INEP, Zemun, Yugoslavia). Results are expressed as antibody titers that bind radioactively labeled thyroglobulin, standardized as follows: below 1:100 - negative; 1:100 to 1:1000 - weakly positive; 1:1000 to 1: 5000 - positive and above 1: 5000 - strongly positive. Presence of anti-sperm antibodies was determined in sera of both partners using a mixed agglutination reaction (MAR, Comhaire *et al.* 1987) and agglutination reaction according to Kibrick *et al.* (1952) and Husted and Hjort 1974) at the Institute for Immunology and Virology Torlak, Belgrade, Yugoslavia. Presence of anti-sperm antibodies in the serum or semen induced agglutination in both tests, which was evaluated microscopically. Sampling for determination of anti-sperm antibodies was not timed in relation to the menstrual cycle.

Results

The control group was referred to as 'fertile' (n=27). The 'unexplained infertility' group (n=42) was a subgroup of the total 'infertile' group (n=65) of patients negative for ASA and with positive postcoital test. Antisperm antibodies were present in 9 out of 65 infertile couples studied (13.8 %). Four of the women were positive for ASA. In one couple, ASA was present in both partners.

Results of the postcoital test were negative in 13 women (20 % of total infertile). In all women that were positive for ASA (n=4) the postcoital test was negative. The test was also negative in women whose partners were positive for ASA (7 out of 9), with the exception of two women that were positive for postcoital test. Of the 13 women with negative postcoital test, which were negative for ASA, and whose partners were also negative for ASA, one woman (7.7 %) had elevated aCL. When nine women negative for ASA, whose partners were positive for ASA, were considered as a group, one third (33 %) was found to have elevated aCL.

The results of aCL and TgAt for the three major groups of women are presented in Tables 1 and 2, respectively. The sera from the control group subjects were found to be below the negative cut-off level for aCL antibodies (<10 GPL Units, 100 %). In the group of total infertile patients elevated aCL antibodies were detected in 21.5 %, the majority (15.4 %) belonging to the group with unexplained infertility, 1.5 % from the group with negative postcoital test, and 4.6 % from the group with partner positive for ASA. Anticardiolipin antibodies were not elevated in four women positive for ASA. The results of TgAt in the same sera (Table 2) showed a small increase in the occurrence of TgAt between the group of fertile (18.5 %) and total infertile (20 %) women. In the group of unexplained infertility, the incidence of TgAt was further increased to 21.4 % of cases.

Table 1. Presence of anticardiolipin IgG antibodies (GPL IU/ml) in sera of fertile and infertile women.

Subject		GPL IU/ml				Positive	
Group	n	<10	10-25	25-60	>60	Within group	Of total infertile
Fertile	27	27 (100 %)	0	0	0	0/27 (0 %)	-
Infertile	65	51 (78.5 %)	13	1	0	14/65 (21.5 %)	14/65 (21.5 %)
Unexplained infertility	42	32 (76.2 %)	9	1	0	10/42 (23.8 %)	10/65 (15.4 %)

Table 2. Presence of antithyroglobulin antibodies in sera of fertile and infertile women.

Subject Group	n	TgAt titer				Positive	
		< 1:100	1:100 - 1:1000	1:1000 - 1:5000	>1:5000	Within group	Of total infertile
Fertile	27	22 (81.0 %)	1	3	1	5/27 (18.5 %)	-
Infertile	65	52 (80.0 %)	9	3	1	13/65 (20 %)	13/65 (20 %)
Unexplained infertility	42	33 (78.6 %)	7	1	1	9/42 (21.4 %)	9/65 (13.8 %)

Discussion

The present results concern the determination of organ-nonspecific anticardiolipin and antithyroid autoantibodies in fertile and infertile women. The results show an increased incidence of aCL antibodies in the group with unexplained infertility, which is in agreement with other published data (Roussev *et al.* 1996, Balasch *et al.* 1996). The incidence of aCL in 21.5 % in infertile patients is comparable to 24 % reported by other authors (Balasch *et al.* 1996). The absence of elevated aCL in the sera of fertile subjects in this study has also been reported for women with children as well as for healthy women that have not yet given birth (Balasch *et al.* 1996). The incidence in the unexplained infertility subjects of 23.8 % was, however, lower than 42 % reported by Roussev *et al.* (1996). There is a possibility that this is a consequence of different concentrations set as negative cut-off levels, namely, some authors have considered sera positive at any detectable concentrations (Sher *et al.* 1994), or differences in standardization of tests used. In this study, however, concentrations of 10 GPL or MPL units were accepted as the borderline in keeping with the recommendation of the manufacturer of the employed assay (based on the mean ± 3 S.D. of 110 subjects within healthy population of both sexes). Of the 14/65 infertile women with aCL antibodies, ten (71 %) came from the group with unexplained infertility.

Presence of TgAt in infertile patients led to the view that they were involved in infertility (van Voorhis and Stovall 1997). There are studies showing that the presence of antithyroid antibodies in 32 % women with abortions pointing to their possible role in early reproductive failure (Singh *et al.* 1995). Our results, however, show only slightly increased incidence of antithyroglobulin antibodies in the group of infertile subjects (20 %), and in patients with idiopathic infertility (21.4 %) compared to the fertile group (18.5 %). In our study, the incidence of elevated TgAt in healthy fertile group of women is considerably higher (18.5 %) than has been reported by Hollowell *et al.* (2002) for United States population (10.4 %). This may be a consequence of subclinically altered thyroid status in some of our control subjects, which could have been missed, since no thyroid function related hormones were determined. In addition, our control group comprised women only, who were reported (Hollowell *et al.* 2002) to have higher TgAt incidence than men, as opposed to the above mentioned study where both sexes were investigated. The different incidence of elevated values for TgAt in the unexplained infertility group in this study as compared to that reported for cases of recurrent abortions mentioned previously, could also reflect the physiological differences between fertilization/implantation problem studied here, and the later maintenance of established pregnancy (Singh *et al.* 1995).

Based on this number of cases, there seems to be no association in the appearance of the types of autoantibodies studied here, since aCL were not detected in infertile patients with ASA, nor were aCL detected in five fertile subjects with an elevated titer of TgAt. Anticardiolipin antibodies, however, were detected in two out of three infertile patients with a high titer of antithyroglobulin antibodies (> 1 : 1000 in one case, and > 1: 5000 in the other). Results of this study on our population of infertile patients confirm the association of aCL and TgAt with unexplained infertility. Investigations involving larger numbers of women may provide better insight into prevalence of organ-specific and other autoantibodies in infertility. Information regarding possible involvement of other autoimmune processes in all the studied subjects may further clarify whether autoantibodies are pathogenetic or just an epiphenomenon. Namely, presence of autoantibodies that

are not specific to components of reproductive system in infertile women are viewed by some authors as an epiphenomenon rather than a factor playing pathogenetic role (Gleicher 1994). In this context, autoantibodies in infertile patients are seen as a part of generalized activation of the immune system (Gleicher 1998), because in these patients, in addition to infertility-associated antibodies, some other classic autoimmune conditions were observed (monoclonal or polyclonal gammapathies, selective IgA deficiencies, and abnormalities of IgG subclasses) (Gleicher *et al.* 1993). In addition, there is a plausible possibility that reproductive failure, in any of its forms, is the first clinical sign of impending autoimmune disease (Gleicher 1999). The mechanism of aPL action pertinent to unexplained infertility is not known. Many mechanisms have been proposed to explain how aPL cause reproductive failure (for review see Kutteh *et al.* 1999), but each of them needs further confirmation.

Abbreviations

APL - antiphospholipid syndrome
aCL - anticardiolipin antibodies
aPL - antiphospholipid antibodies
TgAt - antithyroglobulin antibodies

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