Technical data like assay variance, serum/plasma correlation, performance in internal and external quality assessment schemes and more were evaluated. The consistency of results was found to be highly reliable in the internal quality assessment scheme. The retrospective evaluation of external quality assessment sera (NEQAS) shows a high agreement with the designated targets (details not shown). GMP [3] and quality assurance related aspects are shown schematically and demonstrate the complexity of this field. "GMP in Product Development" (Figure 2) covers elements essential for the way of an "idea" to a high quality final product. "GMP in Production" (Figure 3) documents in a simplified scheme the way from "Raw Material" to "Final Product". The strict use of GMP conform procedures, although being costly and time-consuming, guarantees well-documented products and consistency over time. In addition, the application of these guidelines may increase and ease aspects like standardization and confidence in the test systems used. This is an aspect which is currently being discussed for cardiolipin Abs testing. It should be pointed out that aspects of standardization in the field of autoantibodies testing are gaining more and more importance and interest. Well-documented and controlled production of assays will facilitate standardization and comparability of results easier.

Literature

1. Harris EN, Gharavi, AE Boey, ML et al.(1983)
Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus.
Lancet 2, 1211-1214

2. Cacoub P, Musset L, Piette JC et al. (1997)
Anticardiolipin, Anti-beta2-Glycoprotein I, and Antinucleosome Antibodies in Hepatitis C Virus Infection and Mixed Cryoglobulinemia.
J Rheumatol 24, 2139-2144

3. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm
Link to document: 21CFR820

Multiple autoantibodies associated with autoimmune reproductive failure

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Abstract

Some cases of reproductive failure can be attributed to autoimmune factors. Among these factors, several autoantibodies were found in association with such clinical manifestations, mainly in patients having systemic lupus erythematosus or antiphospholipid syndrome. These autoantibodies include 'classical' anti-phospholipid antibodies such as anti-cardiolipin, anti-β2-glycoprotein-I, anti-phosphatidylserine, and anti-phosphatidyl ethanolamine. There are also some 'non-classical' antiphospholipid antibodies directed to prothrombin, thromboplastin or mitochondrial antibodies of the M5 type, which were also found in patients with reproductive failure. Moreover, animal models as well as some human studies suggest that other autoantibodies, including anti-thyroglobulin, anti-laminin-1, anti-corpus luteum, anti-prolactin, anti-poly (ADP-ribose) and lymphocytotoxic antibodies, also play a role in these clinical manifestations. Even though currently there is not enough data to support a firm association between some of these autoantibodies and reproductive failure, future studies are likely to confirm this and to expand the number of autoantibodies screened in these patients.

Introduction

Reproductive failure complicates many autoimmune diseases such as systemic lupus erythematosus (SLE) and is also a major manifestation of the antiphospholipid syndrome (APS). APS is characterized by various aspects of reproductive failure such as recurrent abortions at various stages, intrauterine growth retardation, pre-eclampsia and, possibly, also infertility [1]. Even though recurrent pregnancy loss and other features of reproductive failure might result from various factors, autoimmune factors provide a major etiology for unexplained recurrent pregnancy loss. Herein we briefly review the association of autoantibodies with reproductive failure with a special emphasis on autoantibodies other than anti-phospholipid antibodies (aPL). This association is evident from either (and sometimes both) human studies or murine models.

'Classical' anti-phospholipid antibodies

aPL are the hallmark of APS, and one of the 2 major clinical manifestations of APS include various aspects of reproductive failure. Thorough and comprehensive discussion of the role of aPL in reproduction is beyond the scope of this review. Nonetheless, some remarks are provided regarding the groups of autoantibodies that are known as the 'classical' aPL:

Anti-cardiolipin

Anti-cardiolipin is probably the most 'classical' aPL, as it is used for the definition of APS. It is highly associated with recurrent pregnancy loss, but also with other manifestations of APS such as focal CNS involvement [2,3]. Animal models support the pathogenic role of anti-cardiolipin in pregnancy [4]. Infusion of anti-cardiolipin antibodies to pregnant mice resulted in a lower fecundity rate, an increased resorption index of embryos, lower number of embryos per pregnancy, and lower weights of embryos and placentae than in the control mice [5]. Following active immunization with a human pathogenic monoclonal IgM aCL (H-3), primary APS developed in BALB/c mice: the mice had high titers of aCL with clinical manifestations typical for APS, mainly obstetric manifestations [6].

Anti-phosphatidylserine

The role of anti-phosphatidylserine antibodies in pregnancy loss is also evident from murine studies. Passive induction of APS has been reported in 2 different studies [7,8]. Moreover, active immunization with phosphatidylserine also led to obstetric clinical manifestations typical of experimental APS:

lower fecundity rate, number of embryos per pregnancy, and weights of embryos and placentae, but higher fetal resorption rate than in the control mice [9].

Anti-phosphatidylethanolamine

There is evidence in some studies that anti-phosphatidylethanolamine is associated with the manifestations of APS such as thrombosis, recurrent fetal loss, neurological features and livedo reticularis [10,11].

Anti-β2-glycoprotein-I

β2-glycoprotein-I is probably the autoantigen in APS. Autoantibodies directed to it are strongly associated with thrombosis, recurrent fetal loss, thrombocytopenia, hemolytic anemia, and heart valve disease [12-14]. Immunization with β2-glycoprotein-I also resulted in the induction of the typical manifestations of obstetric APS [15].

'Non-classical' anti-phospholipid antibodies

In addition to the above-mentioned autoantibodies, there are autoantibodies directed to antigens associated with the coagulation system, and some also serve as co-factors for aPL. There are some reports that also suggest a link to reproductive failure.

Anti-thromboplastin

Thromboplastin is composed of a complex of phospholipids, lipoprotein and cholesterol, and it enables activation of coagulation factor VII upon its binding. The presence of these antibodies has been associated with thrombosis, thrombocytopenia, hemolytic anemia and fetal loss in SLE patients [16]. They are also correlated with lupus anticoagulant and anti-cardiolipin antibody presence.

Anti-mitochondrial antibodies of M5 type

These antibodies are directed towards an unknown antigen located in the inner membranes of mitochondria (50 kDa). They are associated with recurrent fetal loss, hemolytic anemia and thrombocytopenia, and have controversial association with thrombosis [17,18]. They are found in up to 31% of SLE patients, and correlate with the presence of other 'classical' aPL.

Anti-prothrombin

Anti-prothrombin autoantibodies are mainly associated with thrombosis in APS and outside the setting of APS. One example would be IgG anti-prothrombin and anti-β2GPI antibodies measurement at the beginning of a 5-year coronary prevention trial, taken from 106 patients who experienced non-fatal myocardial infarction or cardiac death and 106 subjects without coronary episodes during the follow-up [19]. Anti-prothrombin levels were significantly higher in patients than in controls, and the level of aPT in the highest third of distribution predicted a 2.5-fold increase in the risk of cardiac events. The association of these antibodies with pregnancy loss is still controversial, but a recent study emphasizes a significant association between the presence of anti-prothrombin antibodies and pregnancy loss in APS, especially early pregnancy loss (submitted for publication).

Other autoantibodies

In addition to aPL, there are other autoantibodies reported to be associated with reproductive failure in general or in the setting of APS / SLE.

Anti-thyroglobulin

Some reports support an association between anti-thyroglobulin antibodies and pregnancy loss. Even though thyroid dysfunction can explain this association, the increase in miscarriages cannot always be explained by thyroid dysfunction alone as it can be encountered in the presence of normal thyroid function [20,21]. This suggests that the higher rate of miscarriages observed in women with autoimmune thyroid disturbances primarily reflects an autoimmune phenomenon, rather than or in addition to a consequence of an overt thyroid hormone abnormality. Thus, the presence of antibodies to the thyroid could represent a secondary marker of a predisposition for an autoimmune disease rather then the actual cause of pregnancy loss. We have recently conducted a study in which active immunization of mice with thyroglobulin resulted in the production of anti-thyroglobulin antibodies with an increased rate of fetal resorptions compared with non-immunized mice (submitted for publication). No thyroid pathology could be identified in these mice.

Anti-corpus luteum

Autoantibodies directed to the corpus luteum glycoprotein were found in 22% of female SLE patients who were under 40 years of age. These antibodies were associated with early stages of ovarian dysfunction [22]. This finding might tentatively link these antibodies to infertility, but this assumption should be further confirmed.

Anti-prolactin

Autoantibodies to prolactin have been reported in 5% of SLE patients, but in 41% of SLE patients having also hyperprolactinemia [23]. Their presence is associated with decreased disease activity in lupus, but no clear clinical manifestation. They are associated with increased lymphocyte counts and decreased anti-DNA antibody levels. Even though not determined, the association of these antibodies with hyperprolactinemia might signify decreased conception rates in these patients, as hyperprolactinemia can lead to infertility.

Lymphocytotoxic antibodies

The autoantibodies reacting with lymphocytes are a heterogeneous group of antibodies which target different membranal and intracellular antigens. They are detected in 28-90% of SLE patients, but they also appear in viral diseases, malignancies and rheumatoid arthritis. Many different clinical associations have been described with these autoantibodies including cognitive dysfunction, lupus nephritis, and also spontaneous abortions [24].

Anti-poly (ADP-ribose)

Poly (ADP-ribose) is a branched homopolymer synthesized from the respiratory coenzyme NAD+ at the site of DNA breakage. Autoantibodies against it have been reported in 42-73% of SLE patients, and in these patients they have been associated with obstetric complications including abortion and premature delivery [25].

Anti-laminin

IgG anti-laminin-1 antibodies were found in more than 30% of 177 recurrent aborters, and the levels of these antibodies were higher in recurrent aborters than in healthy pregnant and non-pregnant women. Accordingly, the live birth rate of subsequent pregnancies in IgG anti-laminin-1 positive recurrent aborters was significantly lower than in anti-laminin-1 negative recurrent aborters [26]. Animal models also support such a role for anti-laminin-1. Intravenous injections of rabbit antibodies to laminin induced a high incidence of abortions, fetal death, retroplacental hematomas and hemorrhages in surviving litters. The antibodies were found in the parietal and visceral yolk sac [27]. We recently conducted a study in which immunization with laminin-1 resulted in the production of anti-laminin-1 antibodies and increased fetal resorption rates (submitted for publication). These findings are not surprising as laminins critically contribute to cell differentiation, cell shape and movement, maintenance of tissue phenotypes, and promotion of tissue survival, and hence they are most important during development of early pre-implantation embryos, during the implantation process and during the organogenesis in post-implantation embryos.

Conclusions

Autoimmune factors provide a tool for the differential diagnosis and may be involved in the etiology of reproductive failure. Autoantibodies associated with reproductive failure include mainly aPL, but the above-mentioned data suggest that many other antibodies might be at least a marker of such

clinical presentation. Unfortunately, regarding most of these 'non-classical' and other autoantibodies, there is currently not enough data to suggest a firm association between their presence or elevated levels and clinical manifestations of reproductive failure. Development of animal models along with clinical studies would be able to disclose which autoantibodies could indeed serve as such a marker. Nonetheless, it seems that more autoantibodies would be used in the future for screening patients having currently what is termed 'unexplained' reproductive failure.

References

1. Shoenfeld Y, Sherer Y, Blank M (1998) **Antiphospholipid syndrome in pregnancy – animal models and clinical implications.** Scand J Rheumatol 27 (Suppl 107), 33-36
2. Love PE, Santoro SA (1990) **Antiphospholipid antibodies: anticardiolipin and lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders.** Ann Intern Med 112, 682-98
3. Ninomiya C, Taniguchi O, Kato T, et al. (1992) **Distribution and clinical significance of lupus anticoagulant and anticardiolipin antibody in 349 patients with systemic lupus erythematosus.** Intern Med 31, 194-199
4. Shoenfeld Y, Blank M, Sherer Y (2001) **Induction and treatment of the antiphospholipid syndrome – lessons from animal models.** Eur J Clin Invest 31, 736-740
5. Blank M, Cohen J, Toder V, Shoenfeld Y (1991) **Induction of anti-phospholipid syndrome in naive mice with mouse lupus monoclonal and human polyclonal anti-cardiolipin antibodies.** Proc Natl Acad Sci USA 88, 3069-3073
6. Bakimer R, Fishman P, Blank M, et al (1992) **Induction of primary antiphospholipid syndrome in mice by immunization with a human monoclonal anticardiolipin antibody (H-3).** J Clin Invest 89, 1558-1563
7. Vogt E, Lyden TW, Rote NS (1992) **Monoclonal antiphosphatidylserine antibody induces intrauterine growth retardation in BALB/c mice.** Clin Exp Rheumatol 10, 641
8. Blank M, Tincani A, Shoenfeld Y (1994) **Induction of antiphospholipid syndrome in naive mice with purified IgG antiphosphatidylserine antibodies.** J Rheumatol 21, 100-104
9. Yodfat O, Blank M, Krause I, Shoenfeld Y (1996) **The pathogenic role of anti-phosphatidylserine antibodies: active immunization with the antibodies leads to the induction of antiphospholipid syndrome.** Clin Immunol Immunopathol 78, 14-20
10. Karmochkine M, Berard M, Piette JC, et al. (1993) **Antiphosphatidylethanolamine antibodies in systemic lupus erythematosus.** Lupus 2, 157-160
11. Berard M, Chantome R, Marcelli A, Boffa MC (1996) **Antiphosphatidylethanolamine antibodies as the only antiphospholipid antibodies. I. Association with thrombosis and vascular cutaneous diseases.** J Rheumatol 23, 1369-1374
12. Cabiedes J, Cabral AR, Alarcon-Segovia D (1995) **Clinical manifestations of the antiphospholipid syndrome in patients with systemic lupus erythematosus associate more strongly with anti-β_2-glycoprotein-I than with antiphospholipid antibodies.** J Rheumatol 22, 1899-1906
13. Day HM, Thiagarajan P, Ahn C, et al. (1998) **Autoantibodies to β_2-glycoprotein I in systemic lupus erythematosus and primary antiphospholipid antibody syndrome: clinical correlations in comparison with other antiphospholipid antibody tests.** J Rheumatol 25, 667-674
14. Sanfilippo SS, Khamashta MA, Atsumi T, et al. (1998) **Antibodies to β_2-glycoprotein I: a potential marker for clinical features of antiphospholipid antibody syndrome in patients with systemic lupus erythematosus.** J Rheumatol 25, 2131-2134
15. Blank M, Faden D, Tincani A, et al. (1994) **Immunization with anticardiolipin cofactor (beta-2-glycoprotein I) induces experimental antiphospholipid syndrome in naive mice.** J Autoimmun 7, 441-455
16. Font J, Lopez-Soto A, Cervera R, et al. (1997) **Antibodies to thromboplastin in systemic lupus erythematosus: isotype distribution and clinical significance in a series of 92 patients.** Thromb Res 86, 37-48
17. Tincani A, Meroni PL, Brucato A, et al. (1985) **Anti-phospholipid and anti-mitochondrial type M5 antibodies in systemic lupus erythematosus.** Clin Exp Rheumatol 3, 321-326
18. La Rosa L, Covini G, Galperin C, et al. (1998) **Anti-mitochondrial M5 type antibody represents one of the serological markers for anti-phospholipid syndrome distinct from anti-cardiolipin and anti-β_2 glycoprotein I antibodies.** Clin Exp Immunol 112, 144-151
19. Vaarala O, Puurunen M, Mänttäri M, et al. (1996) **Antibodies to prothrombin imply a risk of myocardial infarction in middle-aged men.** Thromb Haemost 75, 456-459
20. Matalon-Taratkover S, Blank M, Ornoy A, Shoenfeld Y (2001) **The association between anti-thyroid antibodies and pregnancy loss.** Am J Reprod Immunol 45, 72-77
21. Dendrinos S, Papasteriades C, Tarassi K, et al. (2000) **Thyroid autoimmunity in patients with recurrent spontaneous miscarriages.** Gynecol Endocrinol 14, 270-274
22. Pasoto SG, Viana VST, Mendonca BB, et al. (1999) **Anti-corpus luteum antibody: a novel serological marker for ovarian dysfunction in systemic lupus erythematosus?** J Rheumatol 26, 1087-1093
23. Leaños A, Pascoe D, Fraga A, Blanco-Favela F (1998) **Anti-prolactin autoantibodies in systemic lupus erythematosus patients with associated hyperprolactinemia.** Lupus 7, 398-403
24. Breshnihan B, Grigor RR, Oliver M et al. (1977) **Immunological mechanism for spontaneous abortion in systemic lupus erythematosus.** Lancet 2, 1205-1207
25. Kanai Y, Isonishi S, Terashima Y (1989) **Antibody to poly (ADP-ribose) is an indicator of obstetric complications in pregnant patients with systemic lupus erythematosus.** Immunol Lett 21, 217-222
26. Inagaki J, Matsuura E, Nomizu M, et al. (2001) **IgG anti-laminin-1 autoantibody and recurrent miscarriages.** Am J Reprod Immunol 45, 232-238
27. Foidart JM, Yaar M, Figueroa A, et al. (1983) **Abortion in mice induced by intravenous injections of antibodies to type IV collagen or laminin.** Am J Pathol 110, 346-357