Anti-phospholipid Antibodies and Other Immunological Causes of Recurrent Foetal Loss – A Review of Literature of Various Therapeutic Protocols

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Problem
An immune-based aetiology is one of the several accepted causes for recurrent foetal loss (RFL). However, most of the immunological theories have not fulfilled the criteria for causality. This is a review of the various immunological causes of RFL and the outcome of different treatment protocols.

Method of study
Both auto- and alloimmune maternal immunological abnormalities have been proposed to account for foetal loss. Among the autoimmune factors, anti-phospholipid antibodies (APAs) have been demonstrated to be the strongest risk factors for foetal loss, the prevalence of which is as high as 40% in women with RFL. Other autoimmune antibodies implicated in RFL are anti-nuclear antibodies (ANAs), anti-thyroid antibodies and anti-endothelial cell antibodies. The alloimmune factors implicated in pregnancy loss of unknown aetiology include abnormal natural killer (NK) cell activity, alteration in T helper 1 (Th1) and T helper 2 (Th2) ratios, presence of alloimmune antibodies like anti-paternal cytotoxic antibodies, anti-idiotypic antibodies, mixed lymphocyte reaction blocking antibodies and abnormal expression of HLA-G molecules. Management of patients with RFL is mainly based on immunomodulatory (prednisolone, intravenous immunoglobulins, plasma exchange, paternal lymphocyte therapy), anti-aggregation (aspirin) or anti-coagulation (unfractionated or low molecular weight heparin) agents.

Results
Low-molecular-weight heparin with low-dose aspirin has been found to be the most effective treatment for women with APAs and RFL. Differences in dosage, timing of treatment, inclusion criteria, outcome assessment parameters etc. are some of the factors which have resulted in discrepancies in various reports.

Conclusion
Identification of the immunological mechanisms involved in pregnancy loss and the action of different therapeutic reagents is important so that effective therapies can be designed and investigated.

Recurrent foetal loss (RFL) is still a major challenge for physicians as the knowledge about pathophysiology and the mechanism of action of the therapeutic reagents is still limited. During the last 10–15 years, interest in association between acquired and heritable thrombophilia has increased considerably.[1,2] While there is no dispute on the association of anti-phospholipid antibodies (APAs) with pregnancy loss, the data on other immunological factors is still limited and debated.

The major cause of RFL is chromosomal abnormalities. A compilation of the cytogenetic results from 79 published surveys of couples with two or more pregnancy losses comprising 8208 women and 7834 men showed an overall prevalence of chromosomal abnormalities of 2.9%.[3] Among the first trimester abortions, 50–80% of the abortions are reported to be caused by chromosomal anomalies. This figure decreases with advance in gestational age i.e. from 15% in the second trimester to 5% in the third trimester[4] as against 0.6% in the live-born children. Approximately, 50% of all chromosomal abnormalities are balanced reciprocal translocations, 24% Robertsonian translocations, 12% sex chromosomal mosaicisms in female subjects, and the rest consist of deletions, inversions and other abnormalities. Prenatal diagnosis or pre-implantation diagnosis remained the only option for women with abnormal karyotype. Because of the high incidence of karyotype abnormalities in spontaneous abortions, it is often proposed that even in parents with normal karyotype, prenatal diagnosis may be an option for those couples with more than two or more idiopathic pregnancy losses. Management of genetic cause in RFL should include therapy based on the highest level of evidence, genetic counselling, and close monitoring of subsequent pregnancies.

The immunological aetiology of RFL can be either an auto- or alloimmune-mediated affair or both.

Autoimmune factors

Anti-phospholipid Antibodies

Phospholipid molecules are normal components of cell membranes that hold the dividing cells together, which is required for the growth of the developing placenta. In physiological conditions, anionic phospholipids (PL) are located on the inner part of the plasma membrane. Because of the trophoblast tissue remodelling during pregnancy, these PLs get exposed to the external surface, which on subsequent binding to β2 glycoprotein 1 (β2GP1) present on the trophoblast cell membrane acts as a triggering factor for the formation of APAs.[5] APAs are directed against PLs or proteins, most important of which are lupus anti-coagulants (LAs), antibodies against cardiolipin, β2GP1, prothrombin, annexin, phosphatidyl ethanolamine and phosphatidyl inositol.In addition to arterial and/or venous thrombosis as also thrombocytopenia, the cardinal obstetric manifestation of APAs is recurrent pregnancy loss.[6–8]

Several discrete pathological mechanisms of APAs have been described in literature; however, no single mechanism explains the thrombosis induced by APAs. APAs themselves may not cause foetal loss but can damage the inner wall of blood vessels, which leads to the sticking of blood cells at the site of injury resulting in the formation of blood clot. This results in the impairment of blood supply to the foetus and the placenta resulting either in foetal demise or growth retardation.[9]One mechanism suggests that APAs displace annexin V from trophoblast with increased exposure of anionic PL and acceleration of thrombin generation.[10] However, this has been contradicted by a few others.[11]

Studies in mice show that APAs cause pregnancy loss by binding to PLs expressed on the invading trophoblast, thus inhibiting successful embryonic implantation into the endometrium. After the placentation is established, their thrombogenic action leads to decreased placental perfusion and subsequent infarction.[12] This thrombogenic potential will be exaggerated by the known hypercoagulable status that occurs during pregnancy. It has been shown in a recent study that it is the defective trophoblast invasion and not excessive inter-villous thrombosis, which is the most common histological abnormality in APAs associated RFL.[13] Studies have also shown that APAs inhibit the differentiation of extra-villous trophoblasts.[14] In another study it has been reported that cytokines such as tumour necrosis factor-α (TNF-α) and IL-6 may be responsible for the associated thrombosis in women with APAs. Hence, an appropriate cytokine milieu could be responsible for whether the antibodies are pathogenic or merely an epiphenomenon.[15]

Other potential mechanisms of the pro-coagulant effect of APAs are endothelial cell activation; increased adhesion molecule expression; inhibition

of prostacyclin release; increased leucocyte adhesion to endothelial cells; down-regulation of thrombomodulin expression; increased tissue factor (TF) expression and directly stimulated platelet hyperactivity with resultant production of enhanced amounts of the pro-aggregatory molecule of TXA 2. Foetal loss induced by APAs in mice has been reported to be a complement-driven inflammatory condition. Engagement of the complement receptor C5aR on neutrophils induces TF. It is an accepted fact that the most clinically relevant APAs bind to proteins with affinity for PLs. The most important epitope for anti-phospholipid syndrome-related APAs resides on β2GP1. During differentiation to syncytium, the trophoblasts express cell membrane anionic PLs that can bind β2GPI. Adhered β2GPI can be recognized by the antibodies which, interfere with trophoblast cell maturation, resulting in defective placentation.[16–19] The hypercoagulable state in APA patients is associated with alterations in the APC pathway. It is suggested that APAs may impair the protein C anti-coagulant system.[20] Fig. 1 shows some of the proposed mechanisms of APA action in causing foetal loss.

Non-APA Factors

Higher incidence of ANAs in women with RFL indicates that there may be other autoimmune processes that are causative for foetal loss, though the mechanism by which these antibodies can cause foetal loss is not clear.[21] Foetal loss is also found to be associated with increased levels of thyroglobulin and thyroid peroxidase antibodies.[22] As many as 31% of women with RFL are positive for either or both these antibodies. i.e. Higher prevalence of anti-thyroglobulin and anti-thyroperoxidase antibodies, i.e. 10–18%, has also been reported in normally pregnant women without any history of miscarriage or foetal loss.[23,24] It has also been demonstrated that during implantation, there is migration of endovascular trophoblast mononuclear cells,[25] which may get inhibited by anti-endothelial cell antibodies resulting in foetal loss.[26]

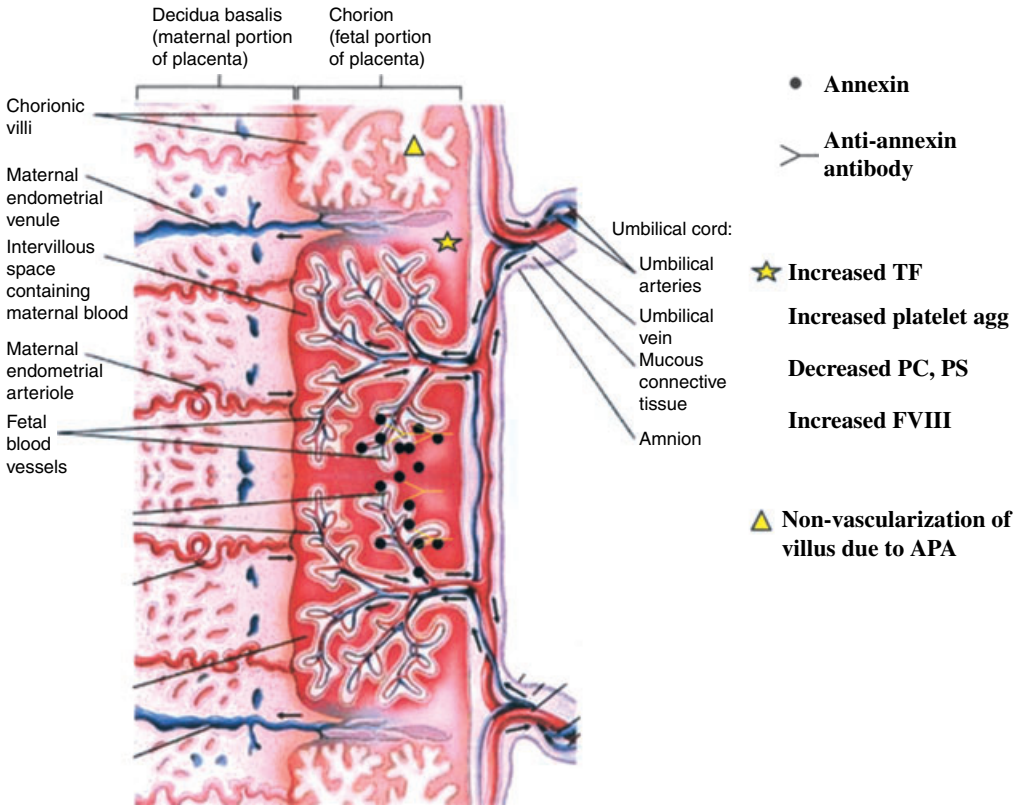

Fig. 1 Different mechanisms of action of anti-phospholipid antibodies in the fetoplacental unit.

Alloimmune Factors

Placenta serves as an immunological barrier during pregnancy in the face of potential maternal immune reactions, which was initially thought to be caused by its non-antigenic nature. However, currently it is clear that trophoblast expresses major histocomatibility antigens on the surface.[27] During pregnancy, maternal immune system recognizes the paternal human leucocyte antigen (HLA) and induces the formation of several alloantibodies [anti-paternal cytotoxic antibodies (APCA), anti-idiotypic antibodies (Ab2) and mixed lymphocyte reaction blocking antibodies (MLR-Bf)], which may coat the trophoblast and protect it from cytotoxic maternal immune response.[28] several reports in the past have shown that reduced or absence of these alloantibodies during pregnancy may cause foetal loss.[29,30]

HLA-G Molecules

Human leucocyte antigen-G is expressed in extravillus trophoblast populations at the materno-foetal interaction surfaces.[31] HLA-G has been shown to be involved in cellular adhesion,[32] indicating that it may play a role in attachment of the blastocyst to the endometrial epithelial cells. Its selective expression by the extra-villous trophoblast cells, which are highly invasive and which eventually invade the uterine arterial system and replace the endothelial cell lining of the blood vessel walls, suggests that it plays an important role in the control of trophoblast invasion.[33] Expression of HLA-G antigens has also been shown to protect cells from NK-cell-mediated lysis, suggesting that its expression may prevent NK cells from attacking the trophoblasts.[34] Although studies on small populations suggested that there are no differences in HLA-G polymorphisms in women with RFL,[35,36] more recent studies have shown an association of the HLA-G*0105N,[37] the HLA-G*0104[38] and the HLA-G*0103[39] with RFL.

Natural Killer (NK) Cell Cytotoxicity

Natural killer cells seem to have a key role in immunosurveillance of the invading trophoblast. However, if activated by TNF-α, NK cells may induce apoptosis in the trophoblast possibly leading to miscarriage.[15]Several studies have shown increased number of CD56+ NK cells in the peripheral blood of women with RFL either prior to or during pregnancy compared with healthy fertile non-pregnant or pregnant controls.[40,41] Studies have also shown that levels of peripheral blood CD56+ cells both prior to and during pregnancy can predict pregnancy outcome in women with RFL.[42,43] In contrast to normal fertile women, where peripheral CD56+ NK cell activity decreases during the first trimester of pregnancy, CD56+ NK cell activity remains high in women with RFL.[41,44] Other studies have shown that the high CD56+ NK cell number and activity is only seen in pregnant RFL women with chromosomally normal foetuses. Whether the presence of high CD56+ levels are a cause or a consequence is not clear.[45] However, another study has shown no differences in the levels of peripheral blood CD56+ cells in women undergoing missed miscarriage with chromosomally normal and abnormal foetuses.[46] In contrast to the increased number of CD56+ cells in peripheral blood, a decreased number of decidual CD56+ NK cells are reported in the placental tissue from spontaneous miscarriages in RFL women compared with tissue from spontaneous miscarriages in women without RFL and women requesting termination.[47,48] A decreased cytotoxic capability of decidual CD56+ NK cells in placental tissue from spontaneous aborters has also been shown, though women with RFL were not included in this study.[49] A report by Lachapelle[50] has shown an increase in the number of CD56+ NK cells in women with RFL as compared with normal pregnant women. The increase or decrease in the number of NK cells in women with RFL at different regions is probably because of the presence of two populations of cells i.e. CD56+ CD16− and CD56+ CD16+. This view is supported by another study wherein an increase in the number of CD16+ cells in early pregnancy loss deciduas has been shown.[51]

Th1/Th2 Balance

The predominant maternal immune response during pregnancy is humoral rather than cell-mediated.[52] Cell-mediated autoimmune diseases such as rheumatoid arthritis are ameliorated during human pregnancy, while antibody-mediated diseases such as systemic lupus erythematosus (SLE) are aggravated, indicating a weakening of the cell-mediated and an enhancement of the antibody response.

Cytokines relevant to pregnancy may be generally divided into two categories, some of them being

harmful for pregnancy by increasing cell-mediated immunity (Th1-type cytokines) which are mainly IL-2, TNF-β, IFN-γ[53] and others exerting a beneficial effect by inhibiting strong cellular responses (Th2 cytokines) which include IL-4, IL-5, IL-10 and IL-13. Both clinical[54,55] and experimental[56] data support the concept that normal pregnancy is a Th2-like phenomenon. There is a clear evidence to prove that the balance between the Th1 and Th2 cytokines at the foeto-maternal interface is extremely important for the foetal survival.

There are several reports of differences in Th1 and Th2 cytokine production by T-cell clones derived from deciduas of women with RFL and normal women undergoing termination of pregnancy.[57] Studies have demonstrated *in vivo* that women with RFL exhibit primarily Th-1 cytokines systemically, whereas healthy women exhibit decreased Th-1 cytokines and increased Th-2 cytokines implying a potential role for a dichotomous T-helper response in the mediation of subsequent reproductive events. The maternal T-helper response appears to operate independently of hormonal factors in influencing the success or failure of human reproduction, as no correlation was observed between serum hormone levels and cytokine levels.[58]

Though there is difference in various factors and cells of the immune system in women with normal pregnancy and women with RFL, yet there is very little data on the mechanism by which these factors act is not very well known. Various hypotheses which have been proposed are increased activity of uterine NK cells and/or macrophages resulting in the attack of the invading trophoblast cells, direct effect of cytokines to cells or the effect of cytokines in causing thrombosis in the placental vasculature.[59]

Therapeutic Protocols

Effective treatment of alleged immune cause of recurrent miscarriage is inhibited for want of more complete knowledge of the underlying pathophysiology. A specific assay to diagnose immune-mediated pregnancy loss and a reliable method to determine which women might benefit from manipulation of the maternal immune system are urgently needed.

Management of APAs during pregnancy in women with history of RFL includes the use of aspirin, heparin, intravenous immunoglobulin (IVIg) and corticosteroids[60–64] either singly or in combination to improve the live-birth rates. A number of randomized and non-randomized clinical trials have analysed these different interventions.[7–10] Although the findings are not consistent because of various limitations of the study groups, yet there has been a move towards use of low molecular weight heparin (LMWH) because of several clinical and practical advantages. It has been advocated because of its presumed mechanism of action against thrombosis and absence of the requirement for frequent monitoring during pregnancy. Additionally it does not cross placenta with no teratogenic effects, has a lower incidence of bone loss and the potential for once-a-day administration.[65,66]

Unfractionated Heparin (UFH) Versus Low Molecular Weight Heparin With or Without Aspirin

Treatment with heparin and/or aspirin is widely accepted as beneficial in APS associated pregnancy loss. Although aspirin alone has shown no clear reduction in pregnancy loss,[67] it does have immunomodulatory potential as it acts as a potent stimulator of IL3. Early clinical studies were based on the assumption that the placental thrombosis was the final pathological manifestation and anti-coagulation would improve the outcome. Now, we know that anti-coagulation mechanism alone cannot explain the beneficial effect of heparin as well as prevention of pregnancy loss. It has been shown previously that LMWH directly interferes with APAs binding to trophoblast cells and is also able to maintain normal trophoblast invasion.[68] β2GP1 has a high affinity for heparin; whether there exists a competition between trophoblast cells and heparin to bind to β2GP1 needs to be analysed. Another study by Bose et al.[69] has shown that UFH can modulate the trophoblast behaviour in a non-thrombotic manner. Treatment with heparin in pregnant patients with APAs also prevents complement activation and protects mice from pregnancy complications induced by APAs and anti-coagulants that do not inhibit complement do not protect pregnancies.[70,71] Both spontaneous and cytokine-boosted abortions in CBA/3 DBA/2 mice were blocked by antibodies to fgl2 prothombinase expressed by cytokine-stimulated vascular endothelial cells and monocytes; *in vivo* antibody depletion of granulocytes also prevented TNF-α1 IFN-γ-induced abortions. Cytokine-triggered thrombotic/inflammatory processes in maternal uteroplacental blood vessels cause abortion.[72]

In vitro studies have shown that both UFH and LMWH have similar effects on APA binding *in vitro*.[73] This finding is significant in that LMWH is reported and tested to be a superior anti-coagulant as compared with UFH. UFH binds to any positively charged protein as a result of the negative charge of the molecule. Because of the low binding to protein in plasma, LMWH has been considered to be a better anti-coagulant than UFH. Further LMWH is a more selective inhibitor of Xa than UFH and has less effect in the inhibition of thrombin. These differences do not seem to appear in an *in vivo* situation while treating patients with APS. There are few exceptions,[64] which shows that the efficacy of both UFH and low-dose aspirin (LDA) is the same as that of LMWH and LDA. Majority of the studies confirm that the most efficacious treatment regimen in women with APAs is a combination of aspirin and LMWH but the question of heparin alone or heparin with aspirin again remains controversial. Table I shows some of the important studies on treatment in women with RFL and APAs with UFH or LMWH with or without LDA.

The randomized trial by Rai et al. compared aspirin (81 mg/day) versus UFH (10,000 U/day) in 90 women.[74] Treatment with LDA in combination with heparin led to a significantly higher rate of live-births (71%) than that achieved with LDA alone (42%; OR 3.37 95% CI 1.40–8.10). Women were excluded if either they or their partner had an abnormal karyotype in this study. A study by Kutteh[75] which included consecutive patients where he assigned aspirin (81 mg/day) or aspirin plus heparin (10,000 U/day), in 50 women with APAs and showed a live-birth rate of 80% versus 40% respectively. Exclusion criteria included women who had SLE, were positive for lupus anti-coagulant, had another abnormal test result that was not corrected either medically or surgically.

The study by Kutteh and Ermel[76]compared the efficacy of lower dose heparin plus aspirin with higher dose heparin for the treatment of APAs associated RFL and found them to be similar with respect to the rate of live-births. However it had serious methodological limitations in that it lacked adequate allocation concealment.

The study by Rosove et al.[77] showed a higher live-birth rate in women treated with a mean daily dosage of 24,700 ± 7400 U (range 10,000–36,000), two doses daily. Fourteen out of 15 pregnancies resulted in live-birth with very few maternal complications. However, the selection of women was based on only a previous pregnancy loss and positive APAs. Thus, they did not fit into the criteria of RFL. Further, the women were not evaluated for karyotypic abnormalities in this study and these results may not be comparable with other studies.

Granger et al.[78] studied 16 women with LMWH (fragmin 5000 U s.c. daily) with LDA and showed a lower success rate i.e. 56% live-births, while in the aspirin-alone group, 76% were successful. This is probably attributable to the selection criteria used for the LMWH group, which was the higher-risk group, being positive for both lupus anti-coagulants (LAs) and anti-cardiolipin antibodies (ACAs). The same authors have subsequently reported that the best outcome of treatment was with women who were positive for ACAs alone. The study by Granger et al. is contradictory to two other studies[31,32] who have compared LDA alone with LDA and heparin for treatment of pregnant women with APAs. Both showed significant improvement in live-birth rate in patients treated with LDA and heparin. However, they differ in the study design and inclusion criteria i.e. Kutteh included patients positive for LAs whereas in the trial by Rai et al., a lower threshold entry criteria for ACA (IgG ≥ 5G PL, IgM > 3 MPL) were used. A major drawback of the study was that no exclusion was made for abnormal karyotype.

Triolo et al.[79] studied 19 women with at least three foetal losses on treatment with LMWH 5700 IU/day along with LDA 75 mg/day and reported a live-birth rate of 84% with no treatment-related complications. Backos et al.[80] treated 150 women with history of RFL and persistent positivity for APAs. He treated patients with heparin (5000 units subcutaneously (s.c.) 12-hourly or enoxaparin 20 mg daily). However, the cut-off levels for ACAs were too low. i.e. ACA IgG > 5 GPU and IgM > 3 MPU. There was a live-birth rate of 71% with 17% gestational hypertension and other associated complications. Cowchock et al.[81] compared the use of low-dose heparin and LDA with prednisolone alone in a multicentre randomized trial showed a similar efficacy with both the treatments except showing a higher association of maternal morbidity in women treated with prednisolone.

There are three studies in which treatment with LDA has been compared with the same with heparin and LDA.[74–76] Two of these studies found evidence that live-birth rate was improved with combination,[74,75] while the third study[76] found that rate of

Table I Studies on Treatment with UFH and/or LMWH Along with LDA in Women with APAs and Foetal Loss

Reference	Total No. pregnancies	No. pregnancy losses prior to therapy	Type of APAs/titer	Event rate *n*/*n* (%)		Dosage	Tx related complications
				UFH	LMWH		
77	14	I or more	LA/ACA	1/15 (6.7)	–	24,700 + 7400 U s.c. total daily dosage twice daily	Increased pre-term and caesarean deliveries
78	16	2 or more	LA/ACA LA positive – DRVVT ratio > 1.10; ACA IgG > 8 GPU/mL and IgM > 5.9 MPU/mL	–	7/16	Fragmin 5000 U s.c. daily and aspirin 75 mg daily	Two developed DVT, pre-term labour in two women
8	26	2 or more	ACA IgM > 11 MPL units or IgG > 30 GPL units or LA	7/26	–	Heparin 10,000 units twice daily s.c. plus aspirin 80 mg/day	Nil
79	19	3 or more			3/19 (16)	Aspirin 75 mg LMWH (seleparina) 5700 IU daily	Mild thrombocytopenia in two women
64	50	3 or more	LA/ACA IgG > 20 GPL or IgM 20 MPL units	5/25	4/25	Aspirin 81 mg daily; enoxaparin 40 mg s.c. daily; UFH 5000 U s.c. twice daily	Nil
76	High dose heparin plus aspirin –25 Low dose heparin and aspirin –25	3 or more than 3	APA IgG > 27 GPL units	High dose heparin plus aspirin – 5/25 Low dose heparin and aspirin – 6/25	–	Heparin 5000 units twice daily (APTT 1.2–1.5 times the baseline-high dose and APTT – upper limit of the normal- low dose) and aspirin 81 mg per day	Nil
74	45	3 or more than 3	ACA IgG > 5 GPL units or IgM > 3 MPL units or LA	13/45	–	Low-dose aspirin (75 mg daily) and 5000 U of UFH s.c. 12 hourly	Nil
75	25	3 or more than 3	ACA or anti-phosphatidyl serine IgG > 27 GPL IgM > 23 MPL; LA excluded	5/25	–	Heparin 5000 U twice daily s.c., 81 mg/day	Nil
80	98	3 or more than 3	ACA		(22%)	Dalteparin 5000 U LDA	Nil
82	150	3 or more	LA IgG > 5 per Liter units IgM > 3 per litre units	43 (29)	Enoxaparin 20 mg	LDA 75 mg daily UFH 5000 U s.c. 12 hourly Enoxaparin 20 mg daily	Gestational hypertension 17% Haemorrhage 7%

foetal loss with LDA alone was low and failed to show any improvement when LDA was used with LMWH. Inclusion criteria were relaxed in the latter study in that women were recruited up to 12 weeks gestation whereas in the former two, all patients were randomized as soon as the pregnancy was detected positive. Kutteh initiated heparin at 5.3 weeks gestation. Thus in the study by Farquharson,[82] about half of the patients were randomized at a more advanced state of gestation. In both the studies by Rai and Farquharson, number of women positive for LAs were similar, however in the study by Kutteh, all women positive for LAs were excluded.

Most of the clinical trials show that LMWH is superior to UFH; as such more and more centres use LMWH with LDA for routine treatment of APAs with previous pregnancy loss. This is mainly based on the fact that most of the settings where UFH and LMWH have been compared, LMWH has been found to be at least as effective as UFH.

Prednisolone and Intravenous Immunoglobulin (IVIg)

Corticosteroids were the first therapeutic reagents proposed for treatment of pregnant women with APAs but were found to have significant morbidity in both the mother (hypertension, gestational diabetes mellitus) and the foetus (pre-mature rupture of membrane, pre-term delivery) without improving pregnancy outcome.[67] IVIg is a fractionated blood product, which is being used for treating a variety of conditions, including spontaneous recurrent miscarriage. Its high costs and short supply necessitate its appropriate applications in specific cases. The mechanism of action of IVIg in the treatment of RFL is multifactorial. They include the modulation of T cells, B cells, NK cells, monocytes and macrophages, down-regulation of antibody production, inhibition of antibody function and modulation of complement activation.[83–86] Sewell and Jolles[87] have proposed that the action may be mediated by the antigen recognizing part of IVIg or because of binding of complement by Fc component of IVIg or because of immunomodulatory substances other than antibody in IVIg preparations. A more recent report suggests the suppression of NK activity by IVIg. The CD56 bright subset of NK cells found at the foeto-maternal surface express receptors for CD200 that bind to CD200R2/3 on antigen-presenting cells, which subsequently activate CD4+ 25+ Treg cells. These Treg cells prevent autoimmune disease and spontaneous abortion.[88,89] Another study by Yamada et al.[90] suggests that there is an immunoregulatory effect on cytokine network, especially the anti-inflammatory effect, which may be important in the therapeutic effects of IVIg. Restoration of Th1/Th2 balance with dominant Th2 may be one of the underlying mechanism of IVIg therapeutic effect in addition to its NK cell-suppression effect.

Steroids and high-dose IVIg have been used successively as reported in several earlier studies. In fact, in most of the trials, these therapies have practically always been combined with anti-thrombotic drugs, so that their specific efficacy cannot be readily assessed. Table II shows results of some of the meta-analyses and clinical trials with IVIg and prednisolone.

Published randomized trials of the use of IVIg and steroids as a treatment for recurrent spontaneous abortion (RSA) have produced conflicting results. In a recent randomized study by Laskin et al.,[91] the combination of steroids and aspirin was no better than placebo, but in this study APA positive patients were mixed with patients positive only for ANA. If only APA positive patients are taken into account, the success rate is 60% in the group receiving steroids and aspirin and 52% in the placebo groups. One meta-analysis of four randomized trials reported a treatment effect of 10%.[92] However, subsequent meta-analysis using both published data and review of individual patient data from six trials indicates that IVIg treatment has no clinically meaningful effect on the live-birth rate.[93] The authors of another randomized trial[94]suggested that IVIg may have a role in the treatment of secondary aborters. A review by Hutton et al.,[95] in which he analysed data from eight trials involving 400 women, IVIg appeared to have a positive effect in secondary aborters while its use in primary aborters was inconclusive.

Triolo et al.[79] compared the efficacy of LMWH and LDA versus IVIg in 40 women and reported a live-birth rate of 84% for the pregnancies in which the women were treated with LMW heparin plus LDA and 57% for those treated with IVIg. Most miscarriages occurred in the first trimester of pregnancy. The difference between the two treatment groups with respect to foetal outcome was more evident during this period. There was no difference between treatments after 13 weeks' gestation. In this regard, it may be hypothesized that heparin contributes to reduction of foetal loss, especially in the first trimes-

Table II Studies on treatment with prednisolone and IVIg in women with APAs and foetal loss

Reference	Study design	Total No. pregnancies	No. pregnancy losses prior to therapy	Type of APAs/-titre	Event rate *n/n* (%)		Dosage
					Prednisolone	IVIg	
92	Prednisolone Plus aspirin versus placebo	101	At least 2 in <32 weeks gestation	Any one of anti-nuclear, anti-DNA, anti-lymphocyte, and ACA, LA	35/101 versus 44/101 in the placebo group	–	Prednisolone 0.8 mg/kg (maximum 60 mg) for the first 4 weeks and then 0.5 mg/kg (maximum 40 mg) plus aspirin 100 mg/day versus placebo
79	IVIg versus LMWH plus aspirin	21	At least 3	LA/ACA	–	12/21 (57) versus 16/19 (84)	IVIg 400 mg/kg/day; aspirin 75 mg/day; heparin 5700 IU/day
97	Prednisolone plus aspirin versus aspirin	12	At least 1 after 12 weeks or at least 2 first trimester losses	ACA/LA	0/12 versus 0/22	–	Prednisolone 20 mg/day increased to a maximum of 40 mg/day depending on the APA titre plus aspirin 81 mg/day versus aspirin 81 mg/day
98	IVIg plus UFH plus aspirin versus UFH plus aspirin	7	–	ACA/LA	0/7 versus 0/9		IVIg 1 g/kg, two days every 4 weeks; aspirin 81 mg/day, heparin 7500 units in the first trimester – 10000 units in the second trimester, twice daily s.c. versus placebo
99	Prednisolone plus aspirin versus untreated	17	>1	ACA	4/17 versus 11/12	–	–
100	Prednisolone plus aspirin	82	>2	LA/ACA	7/29	12/53	–
101	IVIg versus placebo	32	≥2	–		20/32 versus 21/31	500 mg/kg monthly in follicular phase of menstrual cycle up to 6 cycles
102	IVIg versus placebo	47	≥2	–	–	29/47 versus 32/43	500 mg/kg/month every 28 days in the follicular phase until pregnant or for 4 months. After conception 500 mg/kg every 28 days until delivery or up to 32 weeks of gestation
103	IVIg versus placebo	29	≥4 spontaneous miscarriages before 26th gestational week	–	–	16/29 versus 16/29	Treatment started after pregnancy. 0.8 g/kg until gestational week 20, 1 g/kg until 20 26 weeks

ter, by binding to APAs and protecting trophoblast PLs from attack, and producing successful implantation in early pregnancy.

In the study by Silver et al.[96] on treatment efficacy of prednisolone with LDA versus aspirin alone, no perinatal losses were observed in the two study groups. Pre-term delivery was experienced by significantly more patients receiving prednisone plus LDA than aspirin only (8/12 versus 3/22 respectively). In a multicentre, randomized, double-blind pilot study by Branch et al.[97] a comparison of treatment with heparin and LDA plus IVIg with heparin and LDA plus placebo in a group of women who met strict criteria for APS was made. A 100% live-birth rate was observed in both the groups. In this pilot study, IVIg did not improve obstetric or neonatal outcomes beyond those achieved [98] on women with RFL associated with APAs, treatment by prednisolone and LDA therapy from the early gestational period showed that, when started in the early gestational period (prior to 8 weeks gestation), was effective for the achievement of successful pregnancy and the prevention of foetal growth retardation. A prospective, two-centre trial study by Vaquero et al.[99] showed that the live-birth rates with both IVIg and prednisolone and LDA were more or less similar. However, IVIg treatment improved pregnancy outcome with significantly lower pregnancy complication rates, when compared with prednisone plus LDA therapy. In another prospective, randomized, double-blinded, and placebo-controlled study by Stephenson et al.[100], no differences were observed between the IVIg-treated and the placebo groups while Coulam et al.[101] showed a definite enhancement in the percentage of live-births among women experiencing unexplained RFL in IVIg-treated women. Christiansen et al.[94] did not observe any improvement in live-birth rates in IVIg- and placebo-treated women; however, there was a statistically significant improvement in the live-birth rates in secondary aborters when compared with placebo-treated group. In another study, it has been shown that down-regulation of anti-phospholipid antibody production during early pregnancy is associated with favourable pregnancy outcome.[102] Whether addition of IVIg along with heparin and aspirin will improve the pregnancy outcome needs to be looked into.

The timing of treatment is extremely important in analysing the outcome data of treatment of different therapeutic regimens. In a systematic review by Hutton[95] which included eight trials involving 442 women, it was shown that women who were treated during the follicular phase prior to achieving pregnancy experienced less live-births than those who were treated after confirmation of pregnancy. In two studies receiving IVIg prior to conception (n = 102), women had an increased live-birth rate (OR 2.39 95% CI 1.08–5.33, P = 0.03), while in five studies (n = 243), where treatment with IVIg was begun after conception, the live-birth rate did not improve significantly compared with placebo (OR 0.96 95% CI 0.55–1.65, P = 0.87).

Plasma Exchange

Plasmapheresis physically removes antibodies and their end products and thus it has been used for successful treatment of several conditions including syndrome of haemolysis, elevated liver enzymes, and low platelet counts (HELLP), thrombotic thrombocytopenic purpura, and cryoglobulinaemia.[103] Several reports have suggested that plasmapheresis may also be a successful treatment for pregnant women with anti-phospholipid syndrome (APS).[104,105] The last study reported a live-birth rate of 100% in 18 women with documented APS when treated with plasmapheresis and low-dose prednisolone, where other lines of therapy had failed to prevent pregnancy loss with minimal treatment-related complications. Plasmapheresis can be expensive, inconvenient, and may cause several adverse effects such as impaired haemostasis and immunosuppression.[103] Although plasma exchange removes antibodies, a subsequent therapy like IVIg treatment that stops repeat production of antibodies is needed.

Paternal Lymphocyte Therapy

One of the alloimmune causes of RFL is increased sharing of HLA that may prohibit the mother from making APCAs, Ab2 and MLR-Bf. Maternal immunomodulation employing transfusion of paternal leucocytes (lymphocytes) prior to conception has been proposed by several workers.[106–108] The use of third-party donor white cells or trophoblast membranes transfusions have been largely abandoned because of doubts about efficacy.

Results of the randomized and non-randomized studies show that 67% of women with RFL of study group who received paternal lymphocyte immunotherapy showed successful pregnancy outcome in comparison to 36% success in women with RSA of

control group who either received autologous lymphocytes or no therapy.[107] A double-blind placebo-controlled trial in women with unexplained RSA, comparing immunization with paternal maternal (autologous) lymphocytes showed a marginal benefit of paternal lymphocyte therapy over that of maternal lymphocytes.[108]

Progesterone Therapy

The most frequent uses of progesterone are in the prevention of recurrent miscarriage, or in the luteal phase in assisted reproduction programmes, and in threatened pre-term labour. Randomized, controlled trials showed that women who received progesterone were statistically significantly less likely to have recurrent miscarriages before 34 weeks.[109] Progesterone shows favourable immunotolerant actions by stimulating the production of progesterone-induced blocking factor against NK cells, whose activity is crucial to implantation and the interaction between the foetus and the host in early pregnancy.[110,111]

Other Therapeutic Approaches

Newer therapeutic approaches such as monoclonal antibodies like anti-CD20 (rituximab), B-lymphocyte stimulator, B-cell toleragens, which suppress antibody production to domain 1 of β2GP1, antibodies to integrin molecules (efalizumab) are used for various autoimmune disorders including APAs. A wide range of therapies which modulate the immune response have to be explored with least impact on the health of the mother and the foetus.

Summary

Recurrent foetal loss requires aetiological diagnosis for designing appropriate therapeutic protocols. There is evidence now that immunological recognition of pregnancy is important for the maintenance of gestation, and that inadequate recognition of foetal antigens might result in failed pregnancy. Besides APAs and other autoimmune antibodies, some of the alloimmune factors implicated in RFL are: altered Th1/Th2 balance, increased NK cell activity and abnormal HLA-G expression. Maternal immune response is biased toward humoral immunity and away from cell-mediated immunity that could be harmful to the foetus. Yet, the current state of scientific evidence is inadequate. The uncertain aetiology and pathogenesis indicates that treatment is mainly empirical and speculative. Two main treatment modalities exist; one is aimed at reducing the antibodies by either with IVIg, steroids, plasma exchange or donor lymphocyte therapy, while the other includes the use of anti-aggregant or anti-coagulant agents like aspirin and heparin. All these have been used either as monotherapy or in combination. Though these interventions have increased the live-birth rates, there is yet no standard protocol for the use of these therapeutic agents. A number of randomized control trials have been carried out, but they are highly inconsistent and inadequate. The inadequacies mainly are lack of data on the efficacy of different treatment protocols in women positive for APAs other than for LAs and ACAs, lack of consistent data on the same protocol by different authors, lack of uniform primary and secondary outcome assessment, inclusion criteria, dosage and time of starting treatment protocols, positivity and titre of different APAs. Though there is discrepant data on the efficacy of LMWH over UFH, most of the trials have shown superiority of LMWH with LDA in resulting live-births in women with history of RFL. Different studies, regarding the use of IVIg and prednisolone in patients with RFL associated with APAs have resulted in conflicting results. Majority of the studies have shown marginal superiority of IVIg over corticosteroids, though IVIg has resulted in reduced treatment-related complications.

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