

Intravenous immunoglobulin therapy of antiphospholipid syndrome

Y. Sherer, Y. Levy and Y. Shoenfeld

Department of Medicine 'B' and the Research Unit of Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer and Sackler Faculty of Medicine, Tel-Aviv University, Israel

Abstract

Objective. To review the role of intravenous immunoglobulin (IVIg) in antiphospholipid syndrome (APS).

Methods. A literature search was carried out for the immunopathogenesis of APS, laboratory evidence for the beneficial effect of IVIg in APS, and the clinical use of IVIg in APS.

Results. There is both laboratory and clinical evidence for the beneficial role of IVIg in APS. IVIg succeeded in *in vitro* inhibition of anticardiolipin antibodies and lupus anticoagulant, and in the amelioration of experimental APS. Although there are few case reports about IVIg therapy in the haematological manifestations of APS, most of the reports focus on the use of IVIg in the obstetric complications of APS. Hence, in several patient series the use of IVIg either solely or in combination with aspirin/heparin resulted in successful pregnancy outcome in the vast majority of APS patients with recurrent abortions. In addition, IVIg was also beneficial in antiphospholipid antibody-positive patients undergoing *in vitro* fertilization.

Conclusion. APS, an autoimmune disease whose main features are vascular thrombosis and pregnancy morbidity, is a good candidate for immunotherapy with IVIg that contains anti-idiotypes directed towards patients' pathogenic antiphospholipid antibodies. Future research should determine when to use anticoagulation, IVIg or both in the treatment of APS.

KEY WORDS: Antiphospholipid syndrome, Aspirin, Heparin, Intravenous immunoglobulin, Recurrent pregnancy loss.

Intravenous immunoglobulins (IVIg) are currently used in autoimmunity for the treatment of immune thrombocytopenic purpura, Guillain–Barre syndrome, Kawasaki disease, and polymyositis/dermatomyositis. In addition, in several other autoimmune conditions there have been several reports about beneficial responses to IVIg therapy, e.g. systemic lupus erythematosus, vasculitis, factor VIII inhibitors [1–3]. Herein we review the use of IVIg for the treatment of patients with antiphospholipid syndrome (APS).

APS—definition and clinical manifestations

The definition of APS includes the presence of both clinical and laboratory criteria. In order to diagnose APS a patient should have at least one clinical manifestation (vascular thrombosis, pregnancy morbidity) and one typical serological finding [medium to high titres of anticardiolipin antibodies (aCL) or a positive lupus

anticoagulant (LAC) test on two or more occasions at least 6 weeks apart] [4]. The manifestations of this syndrome can further include [5]: venous thrombosis—deep vein thrombosis, pulmonary thromboembolism, Budd–Chiari syndrome, renal vein thrombosis, and ocular thrombosis; arterial thrombosis—stroke, transient ischaemic attack, amaurosis, myocardial infarction, and limb ischaemia; thrombocytopenia and haemolytic anaemia; and other features—epilepsy, migraine, livedo reticularis, chorea, myelopathy, pulmonary hypertension, skin ulcers, Addison's disease, heart valve disease, and ischaemic necrosis of bone. Pregnancy complications of APS include both maternal (pre-eclampsia/eclampsia) and fetal complications (early and late pregnancy loss, intrauterine growth retardation). Similarly, several autoantibodies, other than aCL, might be detected at APS, and these include antibodies directed to phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylglycerol (PG), and phosphatidic acid. These antibodies comprise a group that binds beta-2-glycoprotein-I (beta-2GPI) in association with an acidic phospholipid. Nevertheless, antibodies directed solely to either beta-2GPI or

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Correspondence to: Y. Shoenfeld, Department of Medicine 'B', Chaim Sheba Medical Center, Tel-Hashomer 52621, Israel.

prothrombin are also found in APS. Hence, the syndrome is termed APS rather than aCL syndrome.

The immunopathogenesis of APS

The association of an autoantibody with clinical manifestations of an autoimmune disease does not necessarily implicate that it is of a pathogenic role. Nevertheless, with respect to antiphospholipid antibodies (aPL), there is good evidence that these antibodies are pathogenic in APS, rather than an epiphenomenon. As one of the major clinical manifestations of APS is thrombosis, aPL were found to interact with all the components of the coagulation system [6]. Regarding humoral component involvement, aPL were found to interfere with the activity of protein C, protein S cofactor and antithrombin III [7, 8], and to inhibit kallikrein formation from pre-kallikrein [9]. In addition, as beta-2GPI is a natural anticoagulant that inhibits the prothrombinase activity of platelets [10], binding of aCL to beta-2GPI can disturb its anticoagulant activities and consequently increase thrombin generation. Similarly, this antibody can alter the interference of beta-2GPI with ADP-induced platelet aggregation [11]. Thus, it has been shown that aCL attenuates the inhibitory effect of beta-2GPI on the formation of activated factor X in the presence of activated platelets [12]. aPL may also play a role in platelet activation in different ways: cross-reactivity of aCL with an 80-kDa platelet plasma membrane protein may in turn cause platelet activation [13], and similarly aPL can cause shape change and phosphorylation of 20-kDa protein that in turn may cause enhancement of thrombosis by increased thromboxane A₂ formation, elevated cytosolic calcium concentration, and protein 47-kDa phosphorylation [14]. Furthermore, the interaction of aPL with PS on the surface of activated platelets may cause their subsequent destruction and uptake by the reticulo-endothelial system, which may provide an explanation for the thrombocytopenia characteristic of APS [15]. With respect to the aPL effect on endothelial cells, increased procoagulant activity of endothelial cells and monocytes has been demonstrated in the presence of aPL [16, 17], and these antibodies also enhanced the production of platelet activating factor by endothelial cells [18]. Similarly, decreased production of prostaglandin I₂, which is a potent vasodilator and inhibits platelet aggregation, has been found in association with LAC [19]. Another possible explanation for the thrombotic tendency in APS is an inhibition of anticoagulants by a cross-reaction of aPL with glycosaminoglycans, which are a major component of the non-thrombogenic lining of the vascular endothelium [20].

The most convincing evidence for a pathogenic role of aPL is their ability to induce APS *in vivo* in animal models [21]. Passive induction of APS entails the infusion of either monoclonal or polyclonal aPL to mice in order to create disease manifestations. Infusion of aCL (derived either from mice or humans) to pregnant ICR mice resulted in lower fecundity rate, increased resorption index of embryos, lower number of embryos per

pregnancy, and lower weights of embryos and placentae than in the control mice [22]. Of specific interest is the pathogenic role of anti-PS antibodies demonstrated by passive induction of APS by Blank *et al.* [23]. They demonstrated the ability of polyclonal aPL to cause adverse effects on pregnancy outcome. Moreover, intravascular deposition of IgG and fibrin in the uteroplacental interface, absorption of aCL on placentae, and demonstration of thrombosis in placental vessels, further strengthen the pathogenic role of aPL in APS. Another way to induce APS is via active induction of disease. In short, when an antibody (Ab1) is either injected or generated in response to an autoantigen, its idiotype may stimulate the immune system to produce an antibody against it (Ab2). Consequently, anti-Ab2 (Ab3) may be generated too, and it can simulate the original autoantibody in its binding characteristics. Thus, following active immunization with a human pathogenic monoclonal IgM aCL (H-3), primary APS developed in BALB/c mice: the mice had high titres of aCL with clinical manifestations typical for APS [24]. Similarly, aPL obtained from patients with active APS, as well as monoclonal antibodies generated from mice with secondary systemic lupus erythematosus, succeeded in active induction of primary APS [25–27]. In addition, not only did passive transfer of anti-PS succeed in inducing APS, but active immunization with this antibody also had the same effect [28].

Laboratory evidence for the beneficial effect of IVIg in APS

Whereas other therapeutic options in APS inhibit the consequences of aPL activity (e.g. aspirin and heparin prevent the APS-associated thrombosis), IVIg both inhibits the aPL themselves, and probably decreases their further production. The inhibitory effect of IVIg on aPL, especially aCL, and LAC has been reported by several authors [29–32]. Caccavo *et al.* [29] reported the inhibition of aCL binding to cardiolipin by F(ab')₂ fragment from IVIg in a dose-dependent manner. Similarly, Galli *et al.* [30] demonstrated dose-dependent inhibition of LAC activity in four patients, using either IVIg or F(ab')₂ fragments from it. Finally, partial neutralization of LAC activity was found in 10 of 11 patient sera following incubation with IVIg [31]. These reports suggest the presence of anti-idiotypes to aPL within IVIg preparations. However, whereas anti-idiotypic activity of IVIg is probably the most important mechanism of action of IVIg in the treatment of APS, and it results in short-term neutralization of aPL, it certainly cannot explain the long-term decrease in these autoantibody titres. Hence, another mechanism of action of IVIg in APS involves the inactivation of idiotype-bearing B cell clones, with the subsequent decrease in autoantibody production.

The beneficial effect of IVIg in APS has been well demonstrated in animal models of the disease. Infusion of IVIg to mice in which the disease was induced, as previously discussed, resulted in significantly less fetal

resorptions in comparison with untreated mice [33]. Similar results were obtained from IVIg treatment of experimentally induced systemic lupus erythematosus and primary APS [34]: following treatment with either IVIg or F(ab')₂ fragments, but not with Fc fragments, a complete clinical remission of both diseases was achieved, and it was associated with a decrease in the respective autoantibody titres. As anti-idiotypes to aPL are obviously found within IVIg preparations, the discrepancy between the *in vitro* neutralization of four LAC-positive plasma samples by IVIg, and the neutralization of LAC only in two of these four patients following *in vivo* IVIg administration, led Galli *et al.* [30] to suggest that the clinical efficacy of IVIg in APS should be attributed to other mechanisms rather than anti-idiotypes. This discrepancy can best be explained by the fact that in addition to anti-idiotypes, IVIg preparations also contain aPL [35]. As passive infusion of aPL succeeded in the induction of experimental APS, might IVIg cause an exacerbation of APS rather than clinical improvement of it, or induction of the disease in patients receiving IVIg for another indication? This question was tested by an animal model that compared the immunization of mice with pathogenic monoclonal aCL, and the immunization with IVIg containing aPL [35]. In both cases the mice developed high levels of aPL, but whereas the mice immunized with pathogenic aCL had thrombocytopenia, prolonged APTT and higher resorption rate, the mice immunized with IVIg did not express the clinical manifestations of APS. Therefore, the aPL within IVIg preparations, which also have lower affinity to their respective antigens compared with pathogenic aPL, should be considered as natural autoantibodies.

Clinical use of IVIg in APS

Most of the reports about the use of IVIg in human APS focused on its obstetric complications, mainly recurrent pregnancy loss. Nonetheless, there are a few case reports about treatments of other clinical manifestations of the syndrome (mainly haematological), and these are summarized in Table 1. The first report of IVIg use for recurrent pregnancy loss was probably that of Carreras *et al.* [42] who reported a patient with a history of nine recurrent abortions, who following a 5-day course of 400 mg/kg body weight of IVIg at 17 weeks of gestation, followed by 2-day courses at 22 and 27 weeks of gestation gave birth to a healthy girl at 34 weeks of gestation. This report was followed by several other case reports of successful pregnancy outcome in APS patients with previous habitual abortions [43–46]. However, these cases varied much with respect to dose of IVIg, time of administration, and concomitant therapies used (e.g. heparin, aspirin, prednisone). Of special interest is the report of Ron-el *et al.* [46] of a patient with recurrent fetal loss, in whom a combined treatment with IVIg, aspirin and heparin, succeeded in the delivery of a healthy baby after transfer of frozen embryos.

Naturally, the case series would be much more valuable in the assessment of IVIg efficacy. Kaaja *et al.* [47] reported three APS patients whose history was remarkable for two pulmonary emboli, 13 miscarriages, and one live-born after pregnancy complicated with pre-eclampsia. The treatment of these three women with repetitive doses of IVIg 1 g/kg body weight combined with daily aspirin 75 mg, resulted in three deliveries of healthy babies at term, and one pre-term delivery (at 34 weeks of gestation). The IVIg therapy was also associated with progressive depression of IgG aCL titre. Similar results were reported by Spinnato *et al.* [48] who treated five APS patients (with 17 cumulative previous unsuccessful pregnancies) with monthly courses of IVIg 2 g/kg body weight beginning in the first or early second trimester. Four of them received concomitant heparin and/or aspirin. This treatment resulted in four deliveries of healthy babies at term, and one pre-term delivery (at 32 weeks of gestation). A decrease in IgG aCL titre was observed in three patients. As opposed to the good clinical outcome of pregnancies in the above reports, IVIg therapy in six women with recurrent abortions (five with APS and one with antinuclear antibody) resulted in three complicated twin pregnancies, and three pregnancies with intrauterine growth retardation [49]. However, following each IVIg infusion both IgG and IgM aCL titres significantly decreased. In a larger case series, Valensise *et al.* [50] reported 14 APS patients who received IVIg 1 g/kg body weight every 4 weeks from the 5th to the 33rd week of gestation. Complications occurred in only one patient who had gestational hypertension and abruptio placentae, while in the remaining 13 no maternal, fetal or neonatal complications were found, and the median birth weight was 3433 g. Similarly, a live-birth rate of 84% in 19 pregnancies of 15 patients with APS treated with monthly IVIg courses beginning in the first or early second trimester was reported by Clark *et al.* [51]. Pre-eclampsia occurred in 25% of the pregnancies, 75% of the infants were delivered at 34 weeks of gestation or later, there were no cases of intrauterine fetal growth retardation, and in seven pregnancies the IgG aCL titre decreased. In another report, 38 women with APS and at least three consecutive first trimester spontaneous abortions were treated with IVIg 300 mg/kg body weight at 3-week intervals beginning as soon as pregnancy had been confirmed until the 16th–17th week of pregnancy [52]. In 34 of 38 (89.4%) the pregnancy proceeded beyond the first trimester, while 31 of 38 (81.4%) gave birth to a healthy child at term.

The clinical manifestations associated with aPL progressively expand [53], and one of these clinical entities is infertility, as most studies report an increased prevalence of aPL in infertile women [54]. Sher *et al.* [55] tested whether aPL-positive infertile women undergoing *in vitro* fertilization (IVF) could benefit more from IVIg. Their population study included 89 infertile women who had experienced at least four IVF/embryo transfer failures, of whom 52 were aPL positive and 37 were aPL negative. All 89 patients received daily aspirin 81 mg, daily heparin 10 000 U, and a single infusion of IVIg

TABLE 1. Case reports of IVIg use in non-obstetric manifestations of APS

Reference	Clinical manifestations of APS ^a	Remarks
Sturfelt <i>et al.</i> [36]	Thrombocytopenia	Successful treatment with IVIg after treatment failure with steroids and cytostatic drugs
Cohen and Lui [37]	Haemolytic anaemia, thrombocytopenia, thrombosis, pulmonary haemorrhage	Steroids, plasma exchange, IVIg and cyclophosphamide did not control disease manifestations whereas low-dose aspirin rapidly corrected anaemia and thrombocytopenia
Takagi and Shigekiyo [38]	Bleeding associated with thrombocytopenia	IVIg therapy resulted in disappearance of LAC activity and increase in platelet count
Arnout <i>et al.</i> [39]	Habitual abortions, thrombocytopenia	IVIg therapy resulted in pre-term labour of baby in good health, and elevated platelet count
Vandenbergh <i>et al.</i> [40]	Autoimmune haemolytic anaemia	Several courses of IVIg succeeded in control of haemolysis
Vivaldi <i>et al.</i> [41]	Acquired hypoprothrombinaemia—LAC syndrome	IVIg and steroids resulted in correction of prothrombin time

^aClinical manifestations for which IVIg was administered, not necessarily including the entire clinical spectrum in these patients.

20 g 3–10 days before egg retrieval. Twenty-two of 52 (42%) aPL-positive women achieved live births compared with seven of 37 (19%) of the aPL-negative women ($P = 0.02$). In another study [56], these authors found that among 687 aPL-positive women undergoing IVF, birth rates were significantly higher when aspirin and heparin were administered as compared with no anticoagulation (46% vs 17%). Further, within the group of women who received aspirin and heparin, birth rates were significantly lower for women whose aPL were IgG or IgM directed to PS or PE, compared with women with other types of aPL (17% vs 43%). Interestingly, in this patient subgroup when aPL were directed towards PS or PE, the addition of IVIg to heparin and aspirin in the subsequent IVF cycles was most beneficial, as the birth rates increased from 17 to 41%, whereas in the groups of patients tested positive to other aPL, birth rates did not increase with the addition of IVIg (43% vs 36%) [56].

IVIg therapy in APS—future aims

The therapeutic options for APS progressively increase and include (both experimental and clinical): aspirin, heparin, thromboxane A₂ receptor antagonists, interleukin-3, bromocriptine, anti-CD4 monoclonal antibodies, bone marrow transplantation, anti-idiotypes and IVIg [57]. Future research on the therapy of APS should concentrate on adjustments of the most efficient therapeutic regimen to certain clinical and laboratory manifestations of APS. As thrombosis is probably the most prominent pathological basis of APS, and one can claim that in that case anticoagulation (e.g. aspirin and heparin) would suffice, the report by Sher *et al.* [56] contributes to the understanding that in certain patient subgroups IVIg might have an additive effect to anticoagulation. Moreover, the detrimental effects of aPL should also be attributed to mechanisms other than thrombosis, as aCL was found to have an inhibitory effect on the pulsatility of placental secretion of human chorionic gonadotrophin during early pregnancy [58], and a direct inhibitory effect on trophoblast cells [59],

which may affect the fate of the embryo. Anticoagulation would probably have no effect on these mechanisms of action of aPL, whereas IVIg that neutralizes these antibodies might have. Hence, in the current available therapeutic options, more data should be obtained in order to decide when to use anticoagulation, IVIg or both in the treatment of APS.

References

1. Levy Y, Sherer Y, George J *et al.* Serologic and clinical response to treatment of systemic vasculitis and associated autoimmune diseases with intravenous immunoglobulin. *Int Arch Allergy Immunol* 1999;119:231–8.
2. Levy Y, Sherer Y, Langevitz P *et al.* A study of 20 SLE patients with intravenous immunoglobulin—clinical and serologic response. *Lupus* 1999;8:705–12.
3. Sultan Y, Kazatchkine MD, Nydegger U, Rossi F, Dietrich G, Algiman M. Intravenous immunoglobulin in the treatment of spontaneously acquired factor VIII:C inhibitors. *Am J Med* 1991;91:35S–9S.
4. Wilson WA, Gharavi AE, Koike T *et al.* International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome. *Arthritis Rheum* 1999;42:1309–11.
5. Khamashta MA, Hughes GRV. Phospholipid autoantibodies—cardiolipin. In: Peter JP, Shoenfeld Y, eds. *Autoantibodies*. Amsterdam: Elsevier, 1996:624–9.
6. Aron AL, Gharavi AE, Shoenfeld Y. Mechanisms of action of antiphospholipid antibodies in the antiphospholipid syndrome. *Int Arch Allergy Immunol* 1995;106:8–12.
7. Cariou R, Tobelem G, Bellucci S. Effect of lupus anticoagulant on antithrombotic properties of endothelial cells—inhibition of thrombomodulin-dependent protein C activation. *Thromb Haemost* 1988;60:54–8.
8. Cosgriff TM, Martin BA. Low functional and high antigenic antithrombin III level in a patient with the lupus anticoagulant and recurrent thrombosis. *Arthritis Rheum* 1981;24:94–6.
9. Sanfelippo MJ, Drayna CJ. Prekallikrein inhibition associated with the lupus anticoagulant: a mechanism of thrombosis. *Am J Clin Pathol* 1982;77:275–9.
10. Nimpf J, Bevers EM, Bomans PH *et al.* Prothrombinase activity of human platelets is inhibited by beta 2-glycoprotein-I. *Biochim Biophys Acta* 1986;884:142–9.

11. Nimpf J, Wurm H, Kostner GM. β -glycoprotein I (apo H) inhibits the release reaction of human platelets during ADP-induced aggregation. *Atherosclerosis* 1987;63:109–14.
12. Shi WEI, Chong BH, Hogg PJ, Chesterman CN. Anticardiolipin antibodies block the inhibition by the β_2 -glycoprotein I of the factor Xa generating activity of platelets. *Thromb Haemost* 1993;70:342–54.
13. Kandrotas RJ. Heparin pharmacokinetics and pharmacodynamics. *Clin Pharmacokinet* 1992;22:359–74.
14. Lin YL, Wang CT. Activation of human platelets by the rabbit anticardiolipin antibodies. *Blood* 1992;80:3135–43.
15. Cariou R, Tobelem G, Bellucci S. Effect of lupus anticoagulant on antithrombotic properties of endothelial cells—inhibition of thrombomodulin-dependent protein C activation. *Thromb Haemost* 1988;60:54–8.
16. Oosting JD, Derksen RHW, Blokzijl L, Sixma JJ, de Groot PG. Antiphospholipid antibody positive sera enhance endothelial cell procoagulant activity—studies in a thrombosis model. *Thromb Haemost* 1992;68:278–84.
17. Kornberg A, Blank M, Kaufman S, Shoenfeld Y. Induction of tissue factor-like activity in monocytes by anti-cardiolipin antibodies. *J Immunol* 1994;153:1328–32.
18. Silver RK, Adler L, Hageman JR, Hickman AR. Anticardiolipin antibody-positive serum enhances endothelial cell platelet-activating factor production. *Am J Obstet Gynecol* 1991;165:1748–52.
19. Carreras LO, Vermeylen J, Spitz B, Van Assche A. Lupus anticoagulant and inhibition of prostacyclin formation in patients with repeated abortion, intrauterine growth retardation and intrauterine death. *Br J Obstet Gynaecol* 1981;88:890–4.
20. Kandrotas RJ. Heparin pharmacokinetics and pharmacodynamics. *Clin Pharmacokinet* 1992;22:359–74.
21. Shoenfeld Y, Sherer Y, Blank M. Antiphospholipid syndrome in pregnancy—animal models and clinical implications. *Scand J Rheumatol* 1998;suppl. 107:33–6.
22. Blank M, Cohen J, Toder V, Shoenfeld Y. Induction of anti-phospholipid syndrome in naive mice with mouse lupus monoclonal and human polyclonal anti-cardiolipin antibodies. *Proc Natl Acad Sci USA* 1991;88:3069–73.
23. Blank M, Tincani A, Shoenfeld Y. Induction of antiphospholipid syndrome in naive mice with purified IgG anti-phosphatidylserine antibodies. *J Rheumatol* 1994;21:100–4.
24. Bakimer R, Fishman P, Blank M, Sredni B, Djaldetti M, Shoenfeld Y. Induction of primary antiphospholipid syndrome in mice by immunization with a human monoclonal anticardiolipin antibody (H-3). *J Clin Invest* 1992;89:1558–63.
25. Bakimer R, Blank M, Kosashvilli D *et al*. Antiphospholipid syndrome and the idiotypic network. *Lupus* 1995;4:204–8.
26. Blank M, Krause I, Ben-Bassat M, Shoenfeld Y. Induction of experimental anti-phospholipid syndrome associated with SLE following immunization with human monoclonal pathogenic anti-DNA idiotype. *J Autoimmun* 1992;5:495–509.
27. Cohen J, Bakimer R, Blank M, Valesini G, Shoenfeld Y. Pathogenic serum IgG anticardiolipin antibodies and the idiotypic network. *Ann Rheum Dis* 1993;52:742–8.
28. Yodfat O, Blank M, Krause I, Shoenfeld Y. The pathogenic role of anti-phosphatidylserine antibodies: active immunization with the antibodies leads to the induction of antiphospholipid syndrome. *Clin Immunol Immunopathol* 1996;78:14–20.
29. Caccavo D, Vaccaro F, Ferri GM, Amoroso A, Bonomo L. Anti-idiotypes against antiphospholipid antibodies are present in normal polyspecific immunoglobulins for therapeutic use. *J Autoimmun* 1994;7:537–48.
30. Galli M, Cortelazzo S, Barbui T. *In vivo* efficacy of intravenous gammaglobulins in patients with lupus anticoagulant is not mediated by anti-idiotypic mechanism. *Am J Hematol* 1991;38:184–8.
31. Said PB, Martinuzzo ME, Carreras LO. Neutralization of lupus anticoagulant activity by human immunoglobulin 'in vitro'. *Nouv Rev Fr Hematol* 1992;34:37–42.
32. Matsuda J, Gohci K, Kawasugi K, Tsukamoto M, Saitoh N, Kinoshita T. *In vitro* lupus anticoagulant neutralizing activity of intravenous immunoglobulin. *Thromb Res* 1993;70:109–10 (letter).
33. Bakimer R, Guilburd B, Zurgil N, Shoenfeld Y. The effect of intravenous γ -globulin on the induction of experimental antiphospholipid syndrome. *Clin Immunol Immunopathol* 1993;69:97–102.
34. Krause I, Blank M, Kopolovic J *et al*. Abrogation of experimental systemic lupus erythematosus and primary antiphospholipid syndrome with intravenous gamma globulin. *J Rheumatol* 1995;22:1068–74.
35. Krause I, Blank M, Shoenfeld Y. Anti-DNA and antiphospholipid antibodies in IVIG preparations: *in vivo* study in naive mice. *J Clin Immunol* 1998;18:52–60.
36. Sturfelt G, Mousa F, Jonsson H, Nived O, Thysell H, Wollheim F. Recurrent cerebral infarction and the antiphospholipid syndrome: effect of intravenous gammaglobulin in a patient with systemic lupus erythematosus. *Ann Rheum Dis* 1990;49:939–41.
37. Cohen MG, Lui SF. Multiple complications of the antiphospholipid syndrome with apparent response to aspirin therapy. *J Rheumatol* 1992;19:803–6.
38. Takagi M, Shigekiyo T. Gammaglobulin infusion therapy in a patient with antiphospholipid syndrome. *Jpn J Clin Hematol* 1993;34:496–8.
39. Arnout J, Spitz B, Wittevrongel C, Vanrusselt M, Van Assche A, Vermeylen J. High-dose intravenous immunoglobulin treatment of a pregnant patient with an antiphospholipid syndrome: immunological changes associated with a successful outcome. *Thromb Haemost* 1994;71:741–7.
40. Vandenberghe P, Zachee P, Verstraete S, Demuynck H, Boogaerts MA, Verhoef GE. Successful control of refractory and life-threatening autoimmune hemolytic anemia with intravenous immunoglobulins in a man with the primary antiphospholipid syndrome. *Ann Hematol* 1996;73:253–6.
41. Vivaldi P, Rossetti G, Galli M, Finazzi G. Severe bleeding due to acquired hypoprothrombinemia-lupus anticoagulant syndrome. Case report and review of the literature. *Haematologica* 1997;82:345–7.
42. Carreras LO, Perez GN, Vega HR, Casavilla F. Lupus anticoagulant and recurrent fetal loss: successful treatment with gammaglobulin. *Lancet* 1988;2:393–4.
43. Parke A, Maier D, Wilson D *et al*. Intravenous gammaglobulin, antiphospholipid antibodies and pregnancy. *Ann Intern Med* 1989;110:495–6.
44. Scott JR, Branch DW, Kochenour NK, Ward K. Intravenous immunoglobulin treatment of pregnant patients with recurrent pregnancy loss caused by antiphospholipid antibodies and Rh immunization. *Am J Obstet Gynecol* 1989;159:1055–6.

45. Wapner RJ, Cowchock FS, Shapiro SS. Successful treatment in two women with antiphospholipid antibodies and refractory pregnancy losses with intravenous gammaglobulin infusions. *Am J Obstet Gynecol* 1989;616:1271–2.
46. Ron-el R, Vinder A, Golan A *et al.* The use of intravenous gammaglobulin, heparin and aspirin in the maintenance of pregnancy of freeze thawed embryo in a patient with lupus-type anticoagulant. *Eur J Obstet Gynecol Reprod Biol* 1993;52:131–3.
47. Kaaja R, Julkunen H, Ammala P, Palosuo T, Kurki P. Intravenous immunoglobulin treatment of pregnant patients with recurrent pregnancy losses associated with antiphospholipid antibodies. *Acta Obstet Gynecol Scandinavica* 1993;72:63–6.
48. Spinnato JA, Clark AL, Pierangeli SS, Harris EN. Intravenous immunoglobulin therapy for the antiphospholipid syndrome in pregnancy. *Am J Obstet Gynecol* 1995;172:690–4.
49. Kwak JY, Quilty EA, Gilman-Sachs A, Beaman KD, Beer AE. Intravenous immunoglobulin infusion therapy in women with recurrent spontaneous abortions of immune etiologies. *J Reprod Immunol* 1995;28:175–88.
50. Valensise H, Vaquero E, De Carolis C *et al.* Normal fetal growth in women with antiphospholipid syndrome treated with high-dose intravenous immunoglobulin (IVIg). *Prenatal Diag* 1995;15:509–17.
51. Clark AL, Branch DW, Silver RM, Harris EN, Pierangeli S, Spinnato JA. Pregnancy complicated by the antiphospholipid syndrome: outcomes with intravenous immunoglobulin therapy. *Obstet Gynecol* 1999;93:437–41.
52. Marzusch K, Dietl J, Klein R, Hornung D, Neuer A, Berg PA. Recurrent first trimester spontaneous abortion associated with antiphospholipid antibodies: a pilot study of treatment with intravenous immunoglobulin. *Acta Obstet Gynecol Scandinavica* 1996;75:922–6.
53. Sherer Y, Shoenfeld Y. The antiphospholipid syndrome—an entity to be expanded. *Am J Reprod Immunol* 1999;41:113–6.
54. Sherer Y, Shoenfeld Y. Anti-phospholipid autoantibodies—do they have a pathogenic role in infertility? *Scand J Rheumatol* 1998;suppl. 107:40–3.
55. Sher G, Zouves C, Feiman M *et al.* A rational basis for the use of combined heparin/aspirin and IVIG immunotherapy in the treatment of recurrent IVF failure associated with antiphospholipid antibodies. *Am J Reprod Immunol* 1998;39:391–4.
56. Sher G, Matzner W, Feiman M *et al.* The selective use of heparin/aspirin therapy, alone or in combination with intravenous immunoglobulin G, in the management of antiphospholipid antibody-positive women undergoing *in vitro* fertilization. *Am J Reprod Immunol* 1998;40:74–82.
57. Sherer Y, Blank M, Shoenfeld Y. Immunomodulation of experimental antiphospholipid syndrome. *Scand J Rheumatol* 1998;suppl. 107:48–52.
58. Shurtz-Swirski R, Inbar O, Blank M *et al.* *In vitro* effect of anticardiolipin autoantibodies upon total and pulsatile placental hCG secretion during early pregnancy. *Am J Reprod Immunol* 1993;29:206–10.
59. Chamley LW, Duncalf AM, Mitchell MD, Johnson PM. Action of anticardiolipin and antibodies to β 2-glycoprotein-I on trophoblast proliferation as a mechanism for fetal death. *Lancet* 1998;352:1037–8.