

The preventative role of heparin in antiphospholipid antibody-induced fetal loss

Ronald A Asherson
Rheumatic Diseases Unit,
University of Cape Town
Health Sciences Centre,
Cape Town, The Rosebank
Clinic, Johannesburg,
South Africa
Tel.: +27 117 881 990;
Fax: + 27 117 881 992;
ashron@icon.co.za

Evaluation of: Girardi G, Redecha P, Salmon J: Heparin prevents antiphospholipid-antibody induced fetal loss by inhibiting complement activation. *Nature Med.* 10, 1222–1226 (2004).

Recurrent fetal loss is one of the most important criteria for the diagnosis of antiphospholipid syndrome (APS), along with venous and arterial thromboses, which are usually recurrent. Since the description of the syndrome in 1983, it has been assumed that the mechanism for fetal loss was due to thrombosis. However, this has not been borne out by histopathological studies of many placentae studied from patients with APS. Girardi and colleagues demonstrated previously that complement activation is an essential factor in initiating pregnancy loss, leading to complement C3 activation and deposition with an influx of inflammatory cells into the placenta. This paper demonstrates for the first time that heparin (unfractionated or low molecular weight) prevents complement activation and inhibits the generation of complement split products, thereby protecting mice from antiphospholipid antibody-induced pregnancy complications, an effect that was not seen with the other anticoagulants, such as fondaparinux and hirudin. The authors show clearly that the anticomplementary, rather than the anticoagulant, effect of heparin is responsible for its therapeutic benefit in these patients. The role of complement now assumes major importance in explaining other unusual manifestations of APS as well as its variant, the catastrophic APS.

Pregnancy loss is one of the major criteria for the diagnosis of antiphospholipid syndrome (APS), and it has long been held that impaired blood supply and thrombosis of placental vessels results in placental infarction, with heparin being the standard treatment advised because of its antithrombotic action. The reviewed paper by Girardi and colleagues shows this concept to be erroneous [1]. Several mechanisms are thought to contribute to impairment of the blood supply [2–4]. Annexin V is one of the regulatory proteins of the clotting cascade, which is expressed on endothelial cells in the placenta, as well as on the trophoblast, the precursor to the placenta [5]. Its function is that of a natural anticoagulant at this level, and it performs this by crystallizing over anionic phospholipids, thereby inhibiting coagulation. The antiphospholipid antibodies (aPL) can disrupt this annexin shield, resulting in the generation of thrombin, but immunoglobulin (Ig)G anti-annexin antibodies can also bind to free annexin [6]. Antibodies to B2GP1 also modify trophoblast function and proliferation, thereby altering trophoblastic gonadotropin secretion as a result of the adherence of the B2GP1 to exposed anionic phospholipids [7]. A recent study by Erlebacher and colleagues identified a new link between the immune and reproductive endocrine systems [8].

Systemic immune activation by the CD40 ligand early in pregnancy inhibits the hypothalamic–pituitary–gonadal axis and may cause pregnancy failure. Systemic inflammation induced suppression of cytokine signaling proteins (Socs) and inhibited prolactin signaling and progesterone production. This could be prevented partially by tumor necrosis factor (TNF)- α blockade, implying that this may be an important mechanism for early pregnancy loss, and that TNF blockade may indeed be indicated for patients who experience recurrent early miscarriages.

In 2002, Salmon and colleagues showed that the complement pathway may play an integral and important role in the pathogenesis of fetal loss, totally unrelated to thrombotic or gonadotrophic hormonal blocking effects of the aPL in a mouse model of APS [9]. They demonstrated that activation of the C3 component of complement was needed for fetal loss to occur. They demonstrated subsequently that C5 is also required [10] and suggested that local complement activation could be a mechanism for damage, not only to the trophoblast, but also to the vascular endothelium.

Girardi and colleagues have demonstrated previously that low molecular and unfractionated heparin indeed prevents complement

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activation both *in vivo* and *in vitro* and protects mice from aPL-induced pregnancy complications [1]. By contrast, neither fondaparinux nor hirudin, both solely anticoagulants, inhibit the generation of complement split products or prevent pregnancy losses.

The anticomplementary activity of heparin has been known since 1929 [11]. What Girardi and colleagues have proposed is that preferentially targeted aPL, directed towards the decidua and placenta, leads to the generation of potent anaphylotoxins and mediators of effector cell activation. Recruitment of inflammatory cells accelerates the alternative activation pathway and creates a proinflammatory amplification loop that enhances complement C3 activation and deposition, generates additional C3a and C5a and results in further inflammatory cell migration into the placenta. Inflammatory changes have indeed been described by other authors in placentae taken from women with APS [12,13] and, prior to 2005, elevated levels of complement split products have also been observed in serum studied from patients with APS-related cerebral events [14].

Pierangeli and colleagues, after confirming endothelial cell activation by aPL, confirmed recently that mice deficient in complement components C3 and C5 were resistant to the enhanced thrombosis and endothelial cell activation induced by injected aPL [15]. This again showed, in another model, that aPL were responsible for the activation of complement.

The fact that other manifestations of APS, and particularly some of those seen predominantly in patients with the catastrophic APS (CAPS) [16], may also be related to the role of complement, is evidenced by two further significant papers published recently [17,18].

In a gastrointestinal ischemia–reperfusion (I–R) model, Hart and colleagues found that complement activation played an important role not only in local injury but also in remote injury (e.g., the lung) [17].

They used C1q-deficient (C1qKO), mannose-binding lectin (MBL)-A/C deficient (MBL-null), complement factor 2- and factor B-deficient (C2/fBKO) and wild type (WT) mice. The gastrointestinal injury was followed by 3 h of reperfusion. Local and distant lung injury was induced in the C1qKO and WT mice but not in the C2/fBKO mice. Addition of human C2 in the C2/fBKO mice restored the injury, demonstrating that it is mediated via the lectin and/or classical pathways. The injury significantly

increased serum alanine aminotransferase, gastrointestinal barrier dysfunction and neutrophil infiltration into the lung and gut in the C1qKO and WT mice, but not in the C2/fBKO mice. These researchers demonstrated that C2 and MBL, but not C1q, are necessary for gut injury after gastrointestinal/I–R. Lung injury in mice is MBL and C1q independent but C2 dependent. They suggested a role for ficolins in this model. In addition to MBL, the lectin pathway can be activated by H- and L-ficolin. Although ficolins are synthesized mainly in the liver, L-ficolin is also produced in the lung by alveolar type-11 cells and unciliated bronchial epithelial cells. Importantly, L-ficolin binds to *Escherichia coli* as well as lipoteichoic acid, a cell-wall constituent of Gram-positive bacteria. Gut barrier translocation may lead to bacterial translocation to the lung, resulting in increased pulmonary neutrophil infiltration as a result of lectin complement pathway activation via ficolins. In their second paper, Fleming and colleagues found that aPL could bind to tissues subjected to I–R insult, thus mediating tissue damage [18]. aPL represent members of the natural injury-inducing antibody repertoire of antibodies missing in complement receptor 2-deficient mice. Antibodies to B2GP1 restored both local and remote tissue damage in complement receptor 2-deficient mice. The authors propose that multiple neoantigens are expressed in response to I–R on the endothelial and epithelial surfaces and are recognized by natural autoantibodies, which can fix complement, thereby inducing tissue damage.

This brings us to two important messages:

- APS is comprised of thrombotic and non-thrombotic components. The pathogenesis of the latter manifestations includes a number of pulmonary complications, such as diffuse alveolar hemorrhage (DAH) [19], where there is, in some cases, evidence of the neutrophil pulmonary perivascular infiltrations. These have been previously termed ‘capillaritis’ but now, for the first time, can be identified as being due to local complement/ficolin-induced activation.
- We need to devise other modalities of effective treatment for these nonthrombotic APS manifestations (other than anticoagulation with heparin, which is required to be administered parenterally). These should be directed towards inhibiting complement activation and thus ongoing tissue damage.

Executive summary

- Girardi and colleagues demonstrate clearly a new and important mechanism for fetal loss in patients with antiphospholipid syndrome (APS).
- Activation of complement may play a pivotal role in other manifestations of APS. In particular, the role of ficolins (activated by the lectin pathway) in the pathogenesis of what has been previously termed 'capillaritis' seen in patients with diffuse alveolar hemorrhage, and encountered particularly in the catastrophic variant of APS, is reviewed briefly.
- The high frequency of abdominal symptomatology in catastrophic APS patients and the binding of ficolins to a variety of bacteria present in the bowel may indeed be one of the most important mechanisms contributing to the high mortality and lends credence to the high frequency of triggering infections in this group of patients.
- Peptides directed against the complement activation products C3a and C5a may play an important future role in the therapeutic armamentarium of APS.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

- Girardi G, Redecha P, Salmon J: Heparin prevents antiphospholipid antibody-induced fetal loss by inhibiting complement activation. *Nature Med.* 10, 1222–1226 (2004).
- Mackworth-Young CG, Loizou S, Walport MJ: Antiphospholipid antibodies and disease. *Q. J. Med.* 72, 767–777 (1989).
- Mackworth-Young CG: Antiphospholipid syndrome: multiple mechanisms. *Clin. Exp. Immunol.* 136, 393–401 (2004).
- A comprehensive review of the possible pathogenesis of the thrombotic events seen in patients with antiphospholipid syndrome (APS).
- Gharavi AE, Pierangeli SS, Levy RA, Harris EN: Mechanisms of pregnancy loss in antiphospholipid syndrome. *Clin. Obstet. Gynaecol.* 44, 11–19 (2001).
- Leaders in the field discuss various mechanisms of pregnancy loss in APS.
- Andree HA, Stuart MC, Hermans WT *et al.*: Clustering of lipid-bound annexin V may explain its anticoagulant effect. *J. Biol. Chem.* 267, 7907–7912 (1992).
- Rand JH, Wu XX, Quin AS *et al.*: Human monoclonal antiphospholipid antibodies disrupt the annexin A5 anticoagulant crystal shield on phospholipid bilayers: evidence from atomic force microscopy and functional assay. *Am. J. Pathol.* 161, 56–58 (2003).
- Di Simone N, Meroni PL, Del Papa N: Antiphospholipid antibodies affect trophoblast gonadotropin secretion and invasiveness by binding directly and through adhered β 2 glycoprotein 1. *Arthritis Rheum.* 43, 140–153 (2000).
- Erlebacher A, Zhang D, Parlow AF, Glimbacher LH: Ovarian insufficiency and early pregnancy loss induced by activation of the innate immune system. *J. Clin. Invest.* 114(4), 39–48 (2004).
- Holers VM, Girardi G, Mo L *et al.*: Complement C3 activation is required for antiphospholipid-induced fetal loss. *J. Exp. Med.* 195, 211–220 (2002).
- Girardi G, Berman J, Redecha P *et al.*: Complement C5a receptors and neutrophils mediate fetal injury in the antiphospholipid syndrome. *J. Clin. Invest.* 112, 1644–1654 (2003).
- Ecker E, Gross P: Anticomplementary power of heparin. *J. Infect. Dis.* 44, 250–253 (1929).
- Out HJ, Kooijman CD, Bruinse HW, Derksen RH: Histopathological findings in placentae from patients with intra-uterine fetal death and antiphospholipid antibodies. *Eur. J. Obstet. Gynaecol. Reprod. Biol.* 41, 179–186 (1991).
- Magid MS, Kaplan C, Sammaritano LR: Placental pathology in systemic lupus erythematosus: a prospective study. *Am. J. Obstet. Gynaecol.* 179, 226–234 (1998).
- Davis WD, Brey RL: Antiphospholipid antibodies and complement activation in patients with cerebral ischaemia. *Clin. Exp. Immunol.* 10, 455–460 (1992).
- Pierangeli SS, Girardi G, Vega-Ostertag M, Liu X, Espinola RG, Salmon J: Requirement of activation of complement C3 and C5 for antiphospholipid antibody-mediated thrombosis. *Arthritis Rheum.* 52, 2120–2124 (2005).
- Demonstrates that mice deficient in complement components C3 and C5 are resistant to enhanced thrombosis and endothelial cell activation induced by injected antiphospholipid antibodies (aPL).
- Piette JC, Cervera R, Levy RA, Nasonov EL, Triplett DA, Shoenfeld Y: The catastrophic antiphospholipid syndrome – Asherson's syndrome. *Ann. Med. Interne* 154, 195–196 (2003).
- Hart ML, Ceonzo KA, Shaffer LA *et al.*: Gastrointestinal ischaemia–reperfusion injury is lectin complement pathway dependent without involving C1q. *J. Immunol.* 174, 6373–6380 (2005).
- Complement factor 2 and mannose-binding lectin (MBL), but not C1q, are necessary for gut injury after gastrointestinal ischemia–reperfusion injury. Lung injury is C2 dependent but MBL and C1q independent. The role of L- and H-ficolin activating the lectin pathway is postulated.
- Fleming SD, Egan RE, Chai C *et al.*: Antiphospholipid antibodies restore mesenteric ischaemia reperfusion-induced injury in complement receptor 2/complement receptor 1 - deficient mice. *J. Immunol.* 173, 7055–7061 (2005).
- aPL antibodies can bind to tissues subjected to ischemia–reperfusion injury and mediate tissue damage.
- Asherson RA, Cervera R, Wells A: Diffuse alveolar hemorrhage: a non-thrombotic antiphospholipid lung syndrome? *Semin. Arthritis Rheum.* In Press (2006).
- Diffuse alveolar hemorrhage is one of the important manifestations of the catastrophic APS and may be related to complement activation occurring in the lung secondary to gut barrier translocation of organisms activating L-ficolin synthesized by alveolar and bronchial epithelial cells.

Affiliation

- Ronald A Asherson, MD (Hon), FRCP, MD, FACP, FCP (SA), FACR
Rheumatic Diseases Unit, University of Cape Town Health Sciences Centre, Cape Town, The Rosebank Clinic, Johannesburg, South Africa
Tel.: +27 117 881 990;
Fax: +27 117 881 992;
ashron@icon.co.za