

Role of Tissue Factor in the Maternal Immunological Attack of the Embryo in the Antiphospholipid Syndrome

Guillermina Girardi

© Humana Press Inc. 2009

Abstract Recurrent fetal loss affects 1–5% of women of childbearing age. Immunological mechanisms may account for 40% of recurrent miscarriages, and in particular, the antiphospholipid syndrome (APS) appears to be implicated in 7–25% of the cases. Because antiphospholipid (aPL) antibodies have thrombogenic properties, fetal loss in patients with APS has been ascribed to thrombosis of placental vessels. However, we have shown that inflammation, specifically activation of complement with generation of the anaphylotoxin C5a, is an essential trigger of fetal injury. Thrombosis and inflammation are linked in many clinical conditions. Tissue factor (TF), the major cellular initiator of the coagulation protease cascade, plays important roles in both thrombosis and inflammation, and its expression is increased in patients with APS. Here we describe how TF, acting as a proinflammatory molecule, induces trophoblast injury and fetal death in a mouse model of APS. Importantly, we will discuss how TF contributes to C5a-induced oxidative burst in neutrophils leading to trophoblasts and fetal injury in APS. The finding that TF is an important effector in aPL-induced inflammation may allow the development of new therapies to abrogate the inflammatory loop caused by tissue factor and improve pregnancy outcomes in patients with aPL antibodies. Statins downregulate TF-induced inflammation and rescued the pregnancies in aPL-treated mice, suggesting they may be a good treatment for women with aPL-induced pregnancy complications.

Keywords Pregnancy loss · Antiphospholipid antibodies · Tissue factor · Neutrophils · Protease-activated receptors

Introduction

The antiphospholipid syndrome (APS) is associated with repeated miscarriages and fetal loss. The pathogenesis of pregnancy failures in APS has been related to the thrombophilic effect of antiphospholipid antibodies (aPL). However, we have shown that inflammation plays a crucial role in fetal injury. Using a mouse model of aPL-induced fetal loss, we identified neutrophils as crucial mediators in fetal death. In this review, we will discuss the role of TF, the major cellular activator of the coagulation cascade, in inflammation and fetal injury in aPL antibody-treated mice. We will describe how TF, acting as a proinflammatory molecule rather than a procoagulant molecule, contributes to neutrophil activation, trophoblast injury and fetal loss in APS. We will also discuss the advantages of targeting TF expression with stains as a new therapeutic strategy to prevent miscarriages.

APL antibodies and pregnancy loss

Since the 1950s, aPL has been associated with recurrent miscarriages [1]. Since then, the association between the presence of aPL and the occurrence of repeated spontaneous abortions has been extensively reported [2–7]. Antiphospholipid antibodies are a leading cause of pregnancy loss and pregnancy-related morbidity. Epidemiologic studies have shown that recurrent miscarriage occurs in 3–5% of couples, that up to 20% of women with recurrent miscarriage have aPL antibodies, and that in approximately

G. Girardi (✉)
Department of Medicine,
Weill Medical College of Cornell University,
New York, NY, USA
e-mail: guillerminagirardi@gmail.com

15% of otherwise apparently normal women, aPL is the sole explanation for recurrent fetal loss [8, 9]. Pregnancy loss is a defining criterion for the APS [10, 11]. APS is characterized not only by recurrent fetal loss but also by vascular thrombosis. The association of aPL antibodies with abnormalities of coagulation in vivo has led to the hypothesis that the cause of pregnancy failure is a coagulopathy within the placenta. Thus, the primary treatment for these patients is anticoagulation with heparin and/or aspirin [12, 13]. Anticoagulation throughout pregnancy is inconvenient, sometimes painful, expensive, and fraught with potential complications, including hemorrhage and osteoporosis. Moreover, anticoagulation is often ineffective. Using a mouse model of aPL-induced pregnancy loss, we demonstrated that inflammation rather than thrombosis plays an important role in the pathogenesis of fetal injury. We identified TF as a crucial mediator in aPL-induced recurrent miscarriages. The newly identified mechanism for fetal injury holds the promise of new, safer and better treatments.

Tissue factor and embryo development: a double edged-sword

TF is best known as the primary cellular initiator of blood coagulation. After vessel injury, the TF-FVIIa complex activates the coagulation protease cascade, which leads to fibrin deposition and activation of platelets. Besides initiating the coagulation process, TF contributes to a variety of biological processes. Recent studies indicate that TF plays a role in embryogenesis, metastasis, and tumor-associated angiogenesis [14–16]. TF is expressed during the early stages of both human and murine embryogenesis and targeted disruption of the murine TF (*mTF*) gene results in embryonic lethality [17–19]. TF null embryos exhibit abnormal development of the yolk sac leading to embryo wasting and death. Vitelline vessels from TF null mice are deficient in smooth-muscle alpha-actin-expressing mesenchymal cells, which participate in organization of the vessel wall. This implies that tissue factor has a role in blood vessel development [19]. However, inducible expression of TF in a variety of pathological conditions, including gram-negative sepsis, atherosclerosis, and cancer, is associated with life-threatening complications [20–22]. TF contributes to inflammation and cell injury by interacting with protease-activated receptors (PARs) [23]. Complexes of TF-FVIIa and TF-FVIIa-factor Xa (FXa) as well as FXa and thrombin induce pro-inflammatory signals by activating (PARs) and inducing the expression of TNF- α , interleukins, and adhesion molecules [24–26]. While TF is required for blood vessel formation during embryogenesis, aberrant TF expression can lead to trophoblast injury and

fetal wastage. In this review, we will describe how TF-dependent signaling in neutrophils is responsible for fetal damage in a mouse model of APS. TF expression on neutrophils and formation of a TF-FVIIa complex increase TF oxidative activity and thus contribute to the immunological attack on the conceptus in the APS.

aPL antibodies and tissue factor

Knowing that aPL antibodies are thrombogenic and TF is the initiator of the coagulation cascade, many groups considered the possibility that increased TF expression is present in patients with APS. Indeed, a number of groups have reported increased expression of TF on monocytes from patients with APS [27–29]. In a recent study, Nojima et al. measured the expression of TF on monocytes from patients with systemic lupus erythematosus and found a strong association between high levels of monocyte TF and the presence of aPL antibodies [30]. A number of in vitro studies support a direct role for aPL in inducing monocyte TF expression. Incubation of normal monocytes with IgG from patients with APS, purified aPL IgG, or monoclonal aPL-induced TF expression and activity [31–33]. In particular, anti- β_2 GPI human monoclonal antibodies derived from APS patients enhance monocyte TF mRNA and activity in a β_2 GPI-dependent fashion [34]. Similarly, aPL antibodies (and specifically anti- β_2 GPI antibodies) have been shown to induce TF, along with inflammatory cytokines and adhesion molecules, on endothelial cells [35–37]. Recently, increased TF activity in carotid artery and peritoneal macrophages was described in a mouse model of aPL-induced thrombosis [38]. Ritis et al. reported that complement activation is involved in aPL-induced TF expression on neutrophils. In this study, complement component C5a through interaction with C5a receptor induced TF synthesis and expression on neutrophils [39].

Tissue factor enhances inflammation in aPL-induced pregnancy loss

Using a mouse model of APS, we demonstrated that inflammation plays a crucial role in fetal injury [40, 41]. Passive transfer of aPL antibodies into mice induced fetal resorption ([40]; Table 1). We found that neutrophil depletion with monoclonal antibodies rescued the pregnancies in aPL-induced treated mice ([40]; Table 1). Knowing that TF expression is a characteristic feature associated with aPL antibodies; we sought to investigate whether TF contributes to aPL-induced fetal loss in mice. Mice treated with aPL antibodies showed strong TF staining throughout the decidua and on embryonic debris. In contrast, mice

Table 1 Different strategies (genetically modified mice, monoclonal antibodies, anticoagulants, and statins) used to identify the role of TF, the source of TF, and the mechanism responsible for placental injury and fetal death in aPL-treated mice

Strategy	Target	Outcome in APS mouse
mAb anti-Gr1	Neutrophils	Pregnancy protection
mAb 1H1	Tissue factor (TF)	Pregnancy protection
Low TF mice	TF	Pregnancy protection
Fondaparinux	Coagulation	Fetal loss
Hirudin	Coagulation	Fetal loss
TF ^{floxed/floxed} /LysM-Cre mice	Tissue factor on neutrophils	Pregnancy protection/Inhibition of neutrophil; activation
mAb 10H10	TF-VIIa signaling	Pregnancy protection/Inhibition of neutrophil activation
mAb 5G9	TF procoagulant activity	Pregnancy protection/Inhibition of neutrophil activation
TF δ CT/ δ CT mice	TF-PAR-2 signaling	Inhibition of neutrophil activation
PAR-2 deficient mice	PAR-2	Pregnancy protection/Inhibition of neutrophil activation
Pravastatin	TF and PAR-2	Pregnancy protection/Inhibition of neutrophil activation
Simvastatin	TF and PAR-2	Pregnancy protection/Inhibition of neutrophil activation

treated with control antibodies displayed weak TF staining, which was restricted to the ectoplacental cone region and intact embryo [42]. Surprisingly, neither increase in fibrin staining nor thrombi was associated with increased TF staining in deciduas from aPL-treated mice [42]. Moreover, we have previously shown that anticoagulation with hirudin and fondaparinux was not sufficient to prevent pregnancy loss in this model ([41]; Table 1). Neither TF increase nor fetal death was observed in mice deficient in complement component C5a receptor (C5aR) treated with aPL ([40, 42]; Table 1), demonstrating the importance of C5a-C5aR interaction in TF expression and fetal death in this model of APS.

To assess the importance of TF in aPL-induced fetal injury, we inhibited TF with a monoclonal anti-mTF antibody 1H1 [42]. TF blockade prevented fetal death in aPL-treated mice ([42]; Table 1). Blockade of TF not only rescued pregnancies, it also diminished aPL-induced complement component C3 deposition and neutrophil infiltration in deciduas [42].

To confirm that TF is required for aPL-induced fetal loss, we studied pregnancy outcome in mice expressing low levels of TF [42]. We found that low TF mice treated with aPL antibodies were protected from fetal loss compared with wild-type mice treated with aPL antibodies ([42]; Table 1). That blockade of TF with antibody 1H1 and genetic reduction of TF prevents inflammation and fetal injury demonstrates that TF is a crucial effector molecule in aPL-induced pregnancy loss.

In addition low TF females mated with wild-type males were protected from aPL-induced pregnancy loss, suggesting that maternal TF is crucial for pathology in this model. To distinguish the role of trophoblasts TF from that of myeloid cells, we performed experiments in TF^{floxed/floxed}/LysM-Cre mice that do not express TF on myeloid cells

[42]. These mice were protected from aPL-induced pregnancy loss, emphasizing the key role of TF in maternal myeloid cells (42, Table 1). TF^{floxed/floxed}/LysM-Cre mice treated with aPL showed normal pregnancies and diminished decidual inflammation compared with wild-type mice, suggesting that TF expression on neutrophils modulates the ability of the neutrophil to induce tissue injury. Indeed, neutrophils from TF^{floxed/floxed}/LysM-Cre mice treated with aPL showed a lower generation of oxidants and less phagocytic capacity than neutrophils from aPL-treated TF^{floxed/floxed} control mice. Less free-radical-mediated lipid peroxidation in deciduas was also observed in TF^{floxed/floxed}/LysM-Cre mice treated with aPL when compared with aPL-treated TF^{floxed/floxed} control mice that express TF, suggesting that TF modulates oxidative burst in neutrophils and placental lipid peroxidation [42].

Collectively, this data demonstrates that TF on maternal neutrophils is critical in the pathogenesis of aPL-induced fetal loss and reveals a functional linkage between complement components, TF and neutrophil activation. C5a-C5aR interaction on neutrophils results in increased TF expression leading to neutrophil activation. Activated neutrophils release reactive oxygen species and proteolytic enzymes leading to decidual damage and fetal wastage. TF acts as an important pro-inflammatory mediator in aPL-induced fetal injury [42].

Activation of neutrophils by the tissue factor—factor VIIa—PAR-2 axis mediates fetal death in APS

Knowing the crucial role of TF and inflammation in aPL-induced fetal injury led us to investigate the mechanism by which TF contributes to inflammation in aPL-induced pregnancy loss. Agonists of PARs, notably of PAR-2,

induce inflammation in many diseases associated with neutrophil infiltration and alterations in epithelial permeability [43–45]. Therefore, TF-mediated signaling through PARs may promote inflammation leading to trophoblast injury and pregnancy loss. In order to investigate the role of PAR-2 in aPL-induced TF-mediated neutrophil activity, we first studied PAR-2 synthesis and expression in neutrophils from aPL-treated mice. FACs analysis revealed increased PAR-2 expression on neutrophils from aPL-treated mice when compared to control antibody-treated mice [46]. A 5-fold increase in PAR-2 mRNA expression was also observed in neutrophils from aPL-treated mice when compared to untreated mice or mice treated with control antibodies [46]. Prompted by our finding that PAR-2 expression was increased in neutrophils from aPL-treated mice and that increased reactive oxygen species (ROS) production and phagocytosis was observed in neutrophils from aPL-treated mice, we determined if PAR-2 signaling was involved in neutrophil activation. For this purpose, we studied neutrophils from PAR-2 deficient (PAR-2^{-/-}) mice. The genetic deletion of PAR-2 dramatically reduced neutrophil activation in aPL-treated mice [46]. ROS production and phagocytosis were significantly reduced in neutrophils from PAR-2^{-/-} mice treated with aPL compared with aPL-treated wild-type mice. Specific monoclonal antibody 10H10 that selectively blocks TF-VIIa signaling through PAR-2, but not antibody 5G9 that only prevents TF procoagulant activity, prevented oxidative burst and increased phagocytosis in mice treated with aPL ([46];

Table 1). These results are in accordance with our previous observation that anticoagulation does not rescue pregnancies in APS [41]. In addition, neutrophils from TF δ CT/ δ CT mice that carry a mutation in the cytoplasmic domain involved in PAR-2 signaling did not show increased oxidative stress and phagocytosis in response to aPL ([46]; Table 1). This data reinforces the idea that TF-FVIIa signaling is required for neutrophil activation and fetal injury in APS.

Accordingly to the diminished neutrophil activity observed in aPL-treated PAR-2 deficient mice, genetic deletion of PAR-2 also reduced inflammation in deciduas and rescued pregnancies in aPL-treated mice ([46]; Table 1). PAR-2-deficient mice were protected from aPL-induced fetal injury. These data demonstrate the importance of TF-FVIIa-PAR2 interaction in neutrophil activation and fetal death in this model of APS.

Statins downregulate TF and PAR-2 and prevent miscarriages in aPL-treated mice

In addition to lipid lowering, statins have shown anti-inflammatory effects [47–49]. It has been demonstrated that statins suppressed TF expression in various cell types [50, 51]. Knowing that TF is a crucial mediator in aPL-induced pregnancy loss and that statins diminish TF expression, we investigated if statins can prevent pregnancy loss in aPL-treated mice. In accordance to our hypothesis, simvastatin

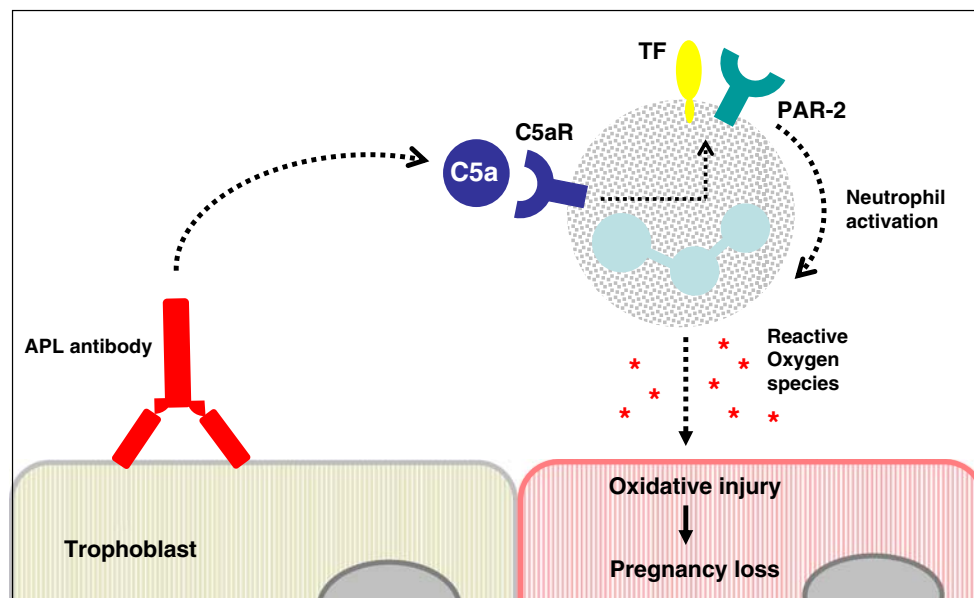


Fig. 1 Proposed mechanism of TF-induced neutrophil activation in fetal death in APS. APL antibodies bind to trophoblasts where they activate the complement cascade leading to the generation C5a. The engagement of C5a with its receptor C5aR on neutrophils results in TF and PAR-2 expression. TF forms a ternary complex with FVIIa

and FXa that activates PAR-2. PAR-2 activation increases neutrophil activation (reactive oxygen species (ROS) production) leading to trophoblast injury and fetal death. Activated neutrophils also release C3, factor B and properdin amplifying the complement cascade, generating more C5a and creating an inflammatory loop

and pravastatin prevented fetal loss in aPL-treated mice ([46]; Table 1). In addition, FACs experiments showed that statins prevented TF and PAR-2 expression on neutrophils from aPL-treated mice [46] and thus prevented neutrophil activation ([46]; Table 1). APL-dependent increase in free-radical-mediated lipid peroxidation in decidual tissue was also ameliorated by treatment with statins [46]. These results suggest that statins prevent aPL-induced neutrophil activation, thus protecting trophoblasts from oxidative damage and rescuing the fetuses.

We recognize that these studies were conducted in mice and that clinical trials are needed to confirm its application to humans. However, given that statins are not major teratogens [51, 52] and considering the beneficial effects of statins in our animal studies, we postulate that statins may be a good treatment for women with aPL-induced pregnancy complications.

Collectively, our studies provide a compelling case that TF has a proinflammatory role in pregnancy loss induced by aPL antibodies. Maternal aPL antibodies bind to the trophoblasts and activate the complement system in the placenta (Fig. 1). Engagement of C5aR triggers expression of TF by neutrophils, resulting in generation of reactive oxygen species, injury to fetal trophoblast cells and ultimately loss of the fetus. TF binds and activates coagulation factors in maternal blood to form the ternary TF/FVIIa/FXa complex. Engagement of PAR2 via this initiation complex of blood coagulation results in neutrophil activation. Statins prevent C5a-induced upregulation of TF and PAR2 expression, thereby inhibiting the release of reactive oxygen species and suppressing amplification of complement activation by factors released from neutrophils. Statins may hold promise as a therapeutic approach to APS-associated pregnancy complications.

References

1. Beaumont JL (1954) Syndrome hemorrhagique acquis du a un anticoagulant circulant. *Sang* 25:1–15
2. Nilsson IM, Astedt B, Hedner U, Berezin D (1975) Intrauterine death and circulating anticoagulant ('antithromboplastin'). *Acta Med Scand* 197:153–159
3. Firkin BG, Howard MA, Radford N (1980) Possible relationship between lupus inhibitor and recurrent abortion in young women. *Lancet* ii:366
4. Loizou S, Byron MA, Englert HJ, David J, Hughes GV, Walport MJ (1988) Association of quantitative anticardiolipin antibody levels with fetal loss and time of loss in systemic lupus erythematosus. *Q J Med* 68:525–531
5. Derksen RH, de Groot PG (2008) The obstetric antiphospholipid syndrome. *J Reprod Immunol* 77(1):41–50
6. Tincani A, Bazzani C, Zingarelli S, Lojacono A (2008) Lupus and the antiphospholipid syndrome in pregnancy and obstetrics: clinical characteristics, diagnosis, pathogenesis, and treatment. *Semin Thromb Hemost* 34(3):267–273
7. Blank M, Shoenfeld Y (2009) Antiphospholipid antibody-mediated reproductive failure in antiphospholipid syndrome. *Clin Rev Allergy Immunol* (in press)
8. Yetman DL, Kutteh WH (1996) Antiphospholipid antibody panels and recurrent pregnancy loss: prevalence of anticardiolipin antibodies compared with other antiphospholipid antibodies. *Fertil Steril* 66:540–546
9. Stephenson MD (1996) Frequency of factors associated with habitual abortion in 197 couples. *Fertil Steril* 66:24–29
10. Wilson WA, Gharavi AE, Koike T, Lockshin MD, Branch DW, Piette JC et al (1999) International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome. *Arthritis Rheum* 42:1309–1311
11. Lockshin MD, Sammaritano LS, Schwartzman SS (2000) Validation of the Sapporo criteria for antiphospholipid antibody syndrome. *Arthritis Rheum* 43:440–443
12. Kutteh WH (1996) Antiphospholipid antibody-associated recurrent pregnancy loss: treatment with heparin and low-dose aspirin is superior to low-dose aspirin alone. *Am J Obstet Gynecol* 174:1584–1589
13. Rai R, Cohen H, Dave M, Regan L (1997) Randomized controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipid antibodies. *J Brit Med* 314:253–257
14. Zhang Y, Deng T, Luther M et al (1994) Tissue factor controls the balance of angiogenic and antiangiogenic properties of tumor cells in mice. *J Clin Invest* 94:1320–1327
15. Soifer SJ, Peters KG, O'Keefe J, Coughlin SR (1994) Disparate temporal expression of the prothrombin and thrombin receptor genes during mouse development. *Am J Pathol* 144:60–69
16. Bugge TH, Xiao Q, Kombrinck KW et al (1996) Fatal embryonic bleeding events in mice lacking tissue factor, the cell-associated initiator of blood coagulation. *Proc Natl Acad Sci USA* 93:6258–6263
17. Parry GC, Erlich JH, Carmeliet P, Luther T, Mackman N (1998) Low levels of tissue factor are compatible with development and hemostasis in mice. *J Clin Invest* 101:560–569
18. Carmeliet P, Mackman N, Moons L et al (1996) Role of tissue factor in embryonic blood vessel development. *Nature* 383:73–75
19. Osterud B, Flæstad T (1983) Increased tissue thromboplastin activity in monocytes of patients with meningococcal infection: related to an unfavorable prognosis. *Thromb Haemost* 49:5–7
20. Edwards RL, Rickles FR, Cronlund M (1981) Abnormalities of blood coagulation in patients with cancer. *J Lab Clin Med* 98:917–928
21. Leatham E, Bath P, Toozé J, Camm A (1995) Increased monocyte tissue factor expression in coronary disease. *Br Heart J* 73:10–13
22. Monroe DM, Key NS (2007) The tissue factor-factor VIIa complex: procoagulant activity, regulation, and multitasking. *J Thromb Haemost* 5:1097–1105
23. Cammer E, Huang W, Coughlin SR (2000) Tissue factor- and factor X-dependent activation of protease-activated receptor 2 by factor VIIa. *Proc Natl Acad Sci USA* 97:5255–5260
24. Ruf W, Dorfleutner A, Riewald M (2003) Specificity of coagulation factor signaling. *J Thromb Haemost* 1:1495–1503
25. Mueller BM, Reisfeld RA, Edgington TS, Ruf W (1992) Expression of tissue factor by melanoma cells promotes efficient hematogenous metastasis. *Proc Natl Acad Sci USA* 89:1–6
26. Cuadrado MJ, López-Pedraza C, Khamashta MA et al (1997) Thrombosis in primary antiphospholipid syndrome: a pivotal role for monocyte tissue factor expression. *Arthritis Rheum* 40:834–841
27. Dobado-Berrios PM, Lopez-Pedraza C, Velasco F, Aguirre MA, Torres A, Cuadrado M (1999) Increased levels of tissue factor mRNA in mononuclear blood cells of patients with primary antiphospholipid syndrome. *Thromb Haemost* 82:1578–1582

28. Reverter JC, Tassies D, Font J et al (1996) Hypercoagulable state in patients with antiphospholipid syndrome is related to high induced tissue factor expression on monocytes and to low free protein S. *Arterioscler Thromb Vasc Biol* 16:1319–1326
29. Nojima J, Masuda Y, Iwatani Y et al (2008) Tissue factor expression on monocytes induced by anti-phospholipid antibodies as a strong risk factor for thromboembolic complications in SLE patients. *Biochem Biophys Res Commun* 365:195–200
30. Reverter JC, Tassies D, Font J et al (1998) Effects of human monoclonal anticardiolipin antibodies on platelet function and on tissue factor expression on monocytes. *Arthritis Rheum* 41:1420–1427
31. Amengual O, Atsumi T, Khamashta MA, Hughes GRV (1998) The role of the tissue factor pathway in the hypercoagulable state in patients with the antiphospholipid syndrome. *Thromb Haemost* 79:276–281
32. Zhou H, Walberg AS, Roubey RAS (2004) Characterization of monocyte tissue factor activity induced by IgG antiphospholipid antibodies and inhibition by dilazep. *Blood* 104:2353–2358
33. Roubey RA, Pratt CW, Buyon JP, Winfield JB (1992) Lupus anticoagulant activity of autoimmune antiphospholipid antibodies is dependent upon beta 2-glycoprotein I. *J Clin Invest* 90:1100–1104
34. Vega-Ostertag M, Casper K, Swerlick R, Ferrara D, Harris EN, Pierangeli SS (2005) Involvement of p38 MAPK in the up-regulation of tissue factor on endothelial cells by antiphospholipid antibodies. *Arthritis Rheum* 52:1545–1554
35. Lopez-Pedraza C, Buendia P, Barbarroja N, Siendones E, Velasco F, Cuadrado MJ (2006) Antiphospholipid-mediated thrombosis: interplay between anticardiolipin antibodies and vascular cells. *Clin Appl Thromb Hemost* 12:41–45
36. Kornberg A, Renaudineau Y, Blank M, Youinou P, Sheffield Y (2000) Anti-beta2-glycoprotein I antibodies and anti-endothelial cell antibodies induce tissue factor in endothelial cells. *Isr Med Assoc J* 2:27–31
37. Vega-Ostertag ME, Ferrara DE, Romay-Penabad Z et al (2007) Role of p38 mutagen-activated protein kinase in antiphospholipid antibody-mediated thrombosis and endothelial cell activation. *J Thromb Haemost* 5:1828–1834
38. Ritis K, Doumas M, Mastellos D et al (2006) A novel C5a receptor-tissue factor cross-talk in neutrophils links innate immunity to coagulation pathways. *J Immunol* 177:4794–4802
39. Girardi G, Berman J, Redecha P et al (2003) Complement C5a receptors and neutrophils mediate fetal injury in the antiphospholipid syndrome. *J Clin Invest* 112:1644–1654
40. Girardi G, Redecha P, Salmon JE (2004) Heparin prevents antiphospholipid antibody-induced fetal loss by inhibiting complement activation. *Nat Med* 10:1222–1226
41. Redecha P, Tilley R, Tencati M et al (2007) Tissue factor: a link between C5a and neutrophil activation in antiphospholipid antibody induced fetal injury. *Blood* 110:2423–2431
42. Bunnett NW (2006) Protease-activated receptors: how proteases signal to cells to cause inflammation and pain. *Semin Thromb Hemost* 32:39–48
43. Chin AC, Lee WY, Nusrat A, Vergnolle N, Parkos CA (2008) Neutrophil-mediated activation of epithelial protease-activated receptors-1 and -2 regulates barrier function and transepithelial migration. *J Immunol* 181:5702–5710
44. Shpacovitch VM, Seeliger S, Huber-Lang M et al (2007) Agonists of proteinase-activated receptor-2 affect transendothelial migration and apoptosis of human neutrophils. *Exp Dermatol* 16:799–806
45. Redecha P, Franzke CW, Ruf W, Mackman N, Girardi G (2008) Neutrophil activation by the tissue factor/Factor VIIa/PA2 axis mediates fetal death in a mouse model of antiphospholipid syndrome. *J Clin Invest* 118:3453–3461
46. Takemoto M, Liao JK (2001) Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. *Arterioscler Thromb Vasc Biol* 21:1712–1719
47. Rosenson R, Tangney C (1998) Antiatherosclerotic properties of statins. *JAMA* 279:1643–1650
48. Paraskevas KI, Stathopoulos V, Mikhailidis DP (2007) Emerging indications for statins: a pluripotent family of agents with several potential applications. *Curr Pharm Des* 13:3622–3636
49. Pierangeli SS, Ferrara DE (2005) More on: fluvastatin inhibits up-regulation of tissue factor expression by antiphospholipid antibodies on endothelial cells. *J Thromb Haemost* 3:1112–1113
50. Kunieda Y, Nakagawa K, Nishimura H et al (2003) HMG CoA reductase inhibitor suppresses the expression of tissue factor and plasminogen activator inhibitor-1 induced by angiotensin II in cultured rat aortic endothelial cells. *Thromb Res* 110:227–234
51. Pollack PS, Shields KE, Burnett DM, Osborne MJ, Cunningham ML, Stepanavage ME (2005) Pregnancy outcomes after maternal exposure to simvastatin and lovastatin. *Birth Defects Res A Clin Mol Teratol* 73:888–896
52. Edison RJ, Muenke M (2005) Mechanistic and epidemiologic considerations in the evaluation of adverse birth outcomes following gestational exposure to statins. *Am J Med Genet A* 135:230–231
53. Contrino J, Hair G, Kreutzer DL, Rickles FR (1996) In situ detection of tissue factor in vascular endothelial cells: correlation with the malignant phenotype of human breast disease. *Nat Med* 2:209–215