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Pravastatin prevents miscarriages in antiphospholipid antibody-treated mice

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Abstract

Miscarriages in patients with antiphospholipid (aPL) antibodies have been attributed to thrombosis of placental vessels. However, we have shown that inflammation plays a crucial role in fetal injury. We identified tissue factor (TF), the major cellular activator of the coagulation cascade, as a key mediator in inflammation and fetal injury in aPL antibody-treated mice. We found that TF in maternal neutrophils was associated with fetal injury. TF expression in neutrophils contributes to the respiratory burst and subsequent trophoblast oxidative injury and pregnancy loss induced by aPL antibodies. We also analysed how TF contributes to neutrophil activation and trophoblast injury in this model. We showed that neutrophils from aPL antibody-treated mice express protease activated receptor 2 (PAR-2) and that stimulation of this receptor leads to neutrophil activation, trophoblast injury and fetal death. Mice deficient in PAR-2 and treated with aPL antibodies exhibited reduced neutrophil activation and normal pregnancies, indicating that PAR-2 plays an important role in the pathogenesis of aPL antibody-induced fetal injury. In addition, we demonstrated that the statins simvastatin and pravastatin downregulate TF and PAR-2 expression in neutrophils and thus prevent pregnancy loss. In summary, this study shows that TF signaling through PAR-2 mediates neutrophil activation and fetal death in antiphospholipid syndrome, and that statins may be an appropriate treatment for women with aPL antibody-induced pregnancy complications.

Keywords: Miscarriages; Antiphospholipid antibodies; Tissue factor; Inflammation; Protease activated receptors; Statins

1. Introduction

Thrombosis in placental vessels is thought to be the cause of fetal death in antiphospholipid syndrome (APS). However, studies performed in our laboratory demonstrated the important role of inflammation rather than thrombosis in fetal demise induced by antiphospholipid (aPL) antibodies (Girardi et al., 2003, 2004; Redecha et al., 2007; Redecha et al., 2008). In aPL antibody-treated mice, increased neutrophil infiltration was observed in the tissues surrounding the dead embryos (Girardi et al., 2003). Moreover, neither fibrin deposition nor thrombi were observed in deciduas from aPL antibody-treated mice (Redecha et al., 2007). In addition, recent human studies showed that inflammatory mechanisms in the placental bed may contribute to APS pregnancy complications, reinforcing the concept of APS as an inflammatory disorder (Stone et al., 2006).

Monocytes from patients with aPL antibodies express tissue factor (TF), and *in vitro* experiments show that monocytes and neutrophils incubated with aPL antibodies express TF (Roubey, 2000; Ritis et al., 2006). The role of TF in blood coagulation is well established. However, TF generates coagulation proteases and activates protease activated receptors (PARs) resulting in inflammation (Randolph, 1998; Macfarlane et al., 2001; Strukova, 2006; Esmon, 2005; Chu, 2006). Here we show that signaling via TF complexed with Factor VIIa

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and PAR-2 (TF–FVIIa–PAR-2) increases neutrophil activation, inducing placental damage and fetal death, emphasizing the proinflammatory effects of TF in fetal death associated with APS. Moreover, we describe how statins that interfere with the TF–FVIIa–PAR-2 signaling axis prevent neutrophil activation and trophoblast injury, rescuing the pregnancies in aPL antibody-treated mice.

2. TF contributes to inflammation and fetal loss in aPL antibody-treated mice

Knowing that TF expression is a characteristic feature associated with aPL antibodies, we sought to investigate whether TF contributes to aPL antibody-induced fetal loss in mice. To test this hypothesis we used a mouse model of aPL antibody-induced pregnancy loss (Girardi et al., 2003, 2004). In this model, passive transfer of human and murine aPL antibodies induces fetal loss and growth restriction (Girardi et al., 2003, 2004). Mice that receive aPL antibodies showed strong TF staining throughout the decidua and on embryonic debris. In contrast, mice treated with control antibodies displayed weak TF staining, which was restricted to the ectoplacental cone region and intact embryo. Surprisingly, neither increase in fibrin staining nor thrombi was associated with increased TF staining in deciduas from aPL antibody-treated mice (Redecha et al., 2007). Neither TF increase nor fetal death was observed in mice deficient in complement component C5a receptor (C5aR) treated with aPL antibodies (Girardi et al., 2003; Redecha et al., 2007), 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 antibody-induced fetal injury, we inhibited TF with a monoclonal anti-murine TF antibody 1H1 (Redecha et al., 2007). TF blockade prevented fetal death in aPL antibody-treated mice (Redecha et al., 2007). Blockade of TF not only rescued pregnancies, but it also diminished aPL antibody-induced complement component C3 deposition and neutrophil infiltration in deciduas.

To confirm that TF is required for aPL antibody-induced fetal loss, we studied pregnancy outcome in mice expressing low levels of TF (low TF mice) (Redecha et al., 2007). We found that low TF mice treated with aPL antibodies were protected from fetal loss compared with wild type mice treated with aPL antibodies (Redecha et al., 2007). 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 antibody-induced pregnancy loss.

Low TF females mated with wild type males were protected from aPL antibody-induced pregnancy loss, suggesting that maternal TF is crucial for pathology in this model (Redecha et al., 2007). To distinguish the role of trophoblast-derived TF from that of myeloid cells, we performed experiments using $TF^{floxed/floxed}$/LysMCre mice that do not express TF on myeloid cells (Redecha et al., 2007). The $TF^{floxed/floxed}$/LysMCre mice were protected from aPL antibody-induced pregnancy loss. The protection from aPL antibody-induced pregnancy loss observed in these mice emphasizes the key role of TF in maternal myeloid cells (Fig. 1A). Moreover, knowing that monocytes are not required for aPL antibody-induced pregnancy loss and that neutrophils from aPL antibody-treated $TF^{floxed/floxed}$/LysM-Cre mice do not express TF allowed us to conclude that TF expression on maternal neutrophils plays a causative and essential role in aPL antibody-induced fetal injury. The $TF^{floxed/floxed}$/LysM-Cre mice treated with aPL antibodies 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 antibodies showed a lower generation of oxidants than neutrophils when compared to $TF^{floxed/floxed}$ control mice. Less free radical-mediated lipid peroxidation in deciduas was also observed in $TF^{floxed/floxed}$/LysM-Cre mice treated with aPL antibodies (Fig. 1B (a)) when compared to aPL antibody-treated $TF^{floxed/floxed}$ control mice (Fig. 1B (b)) that express TF, suggesting that TF modulates oxidative burst in neutrophils and placental lipid peroxidation (Redecha et al., 2007).

Collectively, these data demonstrate that maternal TF on neutrophils is critical in the pathogenesis of aPL antibody-induced fetal loss, and reveal a functional linkage between complement components, TF and neutrophil activation. C5a–C5aR interaction on neutrophils results in increased TF expression. 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 antibody-induced fetal injury (Redecha et al., 2007).

3. Activation of neutrophils by the tissue factor–Factor VIIa–PAR-2 axis mediates fetal death in antiphospholipid syndrome

Knowing the crucial role of TF and inflammation in aPL antibody-induced fetal injury led us to investigate the mechanism by which TF contributes to inflamma-