CASE REPORT

Monocyte activation and acquired autoimmune protein S deficiency promote disseminated intravascular coagulation in a patient with primary antiphospholipid syndrome

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Abstract

Autoimmune protein S (PS) deficiency is a highly thrombotic, potentially life-threatening disorder. Its pathophysiological relevance in the context of primary antiphospholipid syndrome (APS) is unclear. Here, we report the case of a 76-year-old woman, who presented with a painful reticular skin erythema caused by microvascular thromboses. Disseminated intravascular coagulation (DIC) with consumptive coagulopathy was controlled only by continuous anticoagulation. While significantly elevated IgM antibodies to cardiolipin and β_2-glycoprotein-I were consistent with primary APS, a function-blocking PS autoantibody of the IgG isotype was detected. Robust microvesicle (MV)-associated tissue factor (TF) procoagulant activity (PCA) was isolated from patient plasma. Moreover, patient IgG, but not IgM, induced expression of TF PCA and release of TF-bearing MVs by peripheral blood mononuclear cells from healthy donors. In primary APS, induction of monocyte TF in combination with an acquired PS inhibitor may provoke a deleterious imbalance of procoagulant and anticoagulant pathways with evolution of thrombotic DIC.

KEYWORDS
antiphospholipid syndrome, disseminated intravascular coagulation, monocytes, protein S, tissue factor

Essentials

- Deficiency of protein S (PS), a natural regulator of blood clotting, is a risk factor for thrombosis.
- An elderly woman with thrombotic skin vessel occlusions presented with severe PS deficiency.
- Patient immunoglobulins inhibited PS activity and stimulated leukocytes to promote coagulation.
- Combined PS deficiency and leukocyte activation may cause life-threatening thrombosis.

Christina C. Rolling and Florian Langer share senior authorship.

1 | INTRODUCTION

Autoimmune protein S (PS) deficiency is a rare and potentially life-threatening disorder characterized by recurrent thromboembolism due to a defect in the natural anticoagulant protein C–protein S–thrombomodulin (PC-PS-TM) system.[1] Acquired PS inhibitors have been associated with infections,[2,3] multiple myeloma,[4] and other autoimmune diseases, such as the antiphospholipid syndrome (APS)[5] or systemic lupus erythematosus (SLE).[6] In some patients, the antibodies directly interfere with PS anticoagulant activity, but in most cases they are directed against epitopes outside the catalytic domain, resulting in accelerated PS clearance.[7]

APS is the most common acquired thrombophilia and defined by the occurrence of thrombotic or obstetrical complications in the presence of persistent antiphospholipid antibodies (aPL).[8,9] Thrombosis involving all vascular sites can result in a life-threatening, catastrophic condition.[8-10] The pathophysiology of thromboembolism in APS is multifactorial and still incompletely understood, with direct or cofactor-dependent binding of aPL to platelets, leukocytes, or endothelial cells and complement activation playing a role.[9,11]

Although PS antibodies have been identified in individual patients with APS,[5] clinical and laboratory evidence regarding their pathophysiological relevance is still scarce. Here, we provide further insight into the consequences of PS inhibition in the context of aPL positivity.

2 | CASE DESCRIPTION

A 76-year-old woman (150 cm, 48 kg) with a history of recurrent rectal and subcutaneous hemorrhages was referred for the diagnostic workup of an acquired bleeding disorder. Bleeding had initially been attributed to anticoagulation with rivaroxaban 20 mg once daily (OD), which the patient had received for an unprovoked right-sided calf-vein thrombosis 3 months earlier. However, bleeding symptoms had persisted despite cessation of anticoagulation.

At presentation, a highly painful reticular livid skin erythema was found on the patient's trunk (Figure 1A). Histological examination revealed leukocytoclastic vasculitis and microvascular thromboses (Figure 1B). Laboratory findings were consistent with disseminated intravascular coagulation (DIC) and consumptive coagulopathy (Table 1). Treatment of concurrent sigmoid diverticulitis did not resolve DIC, and overt malignancy was excluded. Except for slightly elevated IgM anticardiolipin antibodies (aCL), there was no laboratory evidence for an underlying autoimmune
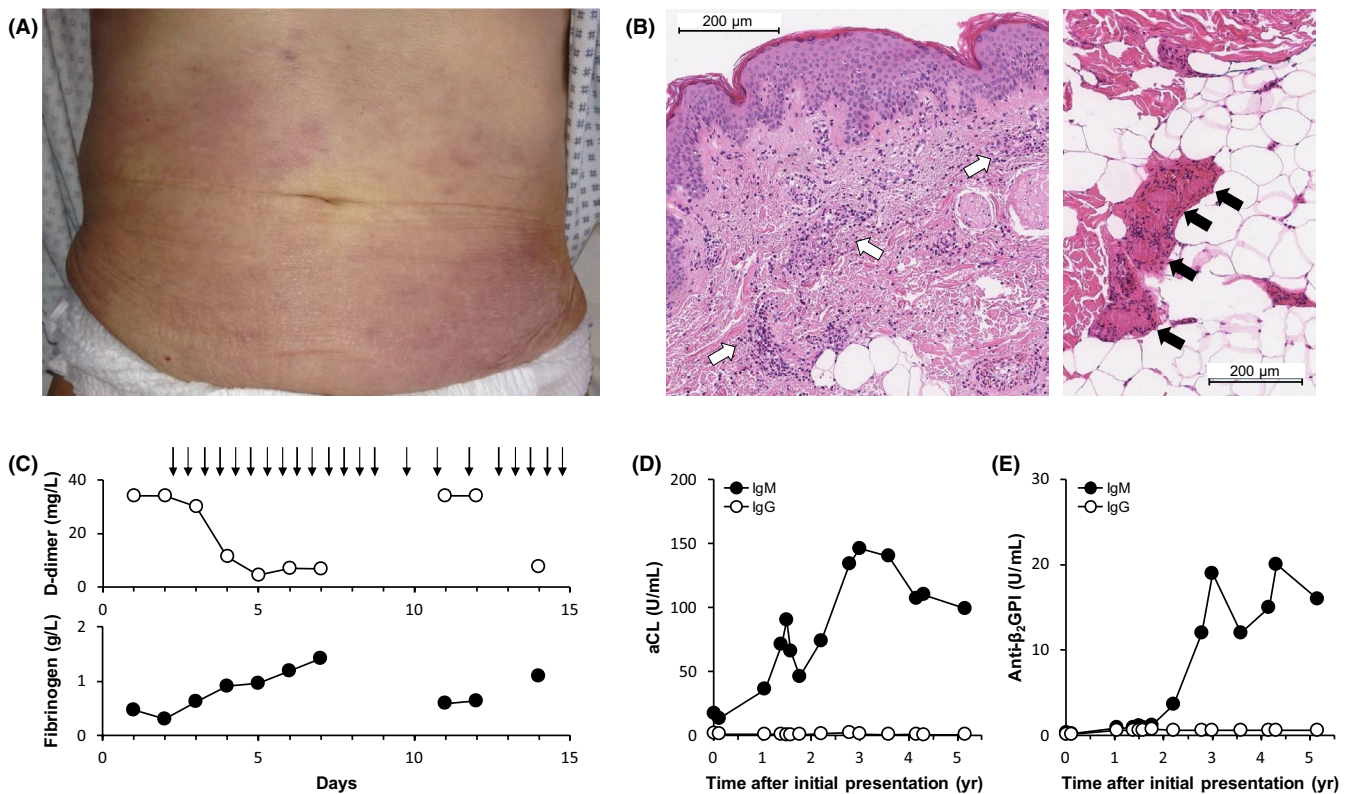

FIGURE 1 Skin manifestations and clinical course of DIC and antiphospholipid antibodies (aPL). (A) At initial presentation, a highly painful reticular livid skin erythema on the patient's trunk indicated microvascular thromboses. (B) Histopathological analysis of a skin biopsy specimen revealed nonspecific leukocytoclastic vasculitis of smaller dermal (left panel) and thrombotic occlusions of larger subcutaneous vessels (right panel). (C) Initial time course of plasma D-dimer and fibrinogen during anticoagulation with the low-molecular-weight heparin enoxaparin. Each arrow indicates administration of 20 mg of enoxaparin. (D, E) The aPL profile was routinely assessed during follow-up. Time courses for cardiolipin antibodies (aCL) (D) and β_2-glycoprotein I-antibodies (anti-β_2GPI) (E) are shown

TABLE 1 Laboratory workup of the patient

	Admission	1.5	3	Reference range
		Time after initial presentation, yr		
Blood counts				
Hemoglobin, g/dL	10.6	12.3	11.7	12.3-15.3
Leukocytes, 1×10^9/L	9.0	5.4	7.0	3.8-11.0
Platelets, 1×10^9/L	166	324	308	150-350
Coagulation parameters				
Prothrombin time, %	45.4	104.2	97.0	80-130
INR	1.52	0.99	1.0	0.85-1.15
aPTT, s	42.5	32.6	32	25-38
Thrombin time, s	45.9	16.5	23	16-22
Fibrinogen, g/L	0.47	2.57	1.89	1.8-4.0
D-dimer, mg/L	>34	1.51	1.69	<0.5
Antithrombin, %	95.0	110.2	116	70-130
PC antigen, %	n.d.	108.2	83.8	65-140
PC activity,[*] %	34.2	40.2	26.0	70-140
Total PS antigen, %	n.d.	73.0	82.0	55-125
Free PS antigen, %	69.2	96.0	80.4	60-130
PS activity, %	21	13.9	<8.0	55-125
Ratio APC resistance	1.7	1.7	1.7	>2.2
Antiphospholipid antibodies				
IgM aCL, U/mL	17	66	146	<10
IgG aCL, U/mL	1.7	<0.5	1.0	<10
IgM anti-β_2GPI, U/mL	0.3	<0.9	19	<7
IgG anti-β_2GPI, U/mL	<0.1	<0.6	<0.6	<7
LA	Negative	Negative	Negative	Negative

aCL, anticardiolipin; anti-β_2GPI, anti-β_2-glycoprotein-I; APC, activated protein C; aPTT, activated partial thromboplastin time; INR, international normalized ratio; LA, lupus anticoagulant; n.d., not determined; PS, protein S.

*Protein C (PC) activity was assessed with a clotting-based assay.

disease (Table 1, Table S1). An empirical short-term course of oral corticosteroids had no effect. Screening for other aPL was negative (Figure S1).

DIC was controlled only by uninterrupted anticoagulation with the low-molecular-weight heparin (LMWH) enoxaparin (Figure 1C). Prolongation of administration intervals resulted in immediate relapse of consumptive coagulopathy. Following DIC stabilization with enoxaparin, the patient was reexposed to rivaroxaban 20 mg OD (Figure S2A), under which the coagulopathy spontaneously relapsed with painfully reduced acral perfusion and elevated plasma D-dimers. Since long-term administration of LMWH was deemed unfeasible, the patient was switched to oral apixaban 5 mg twice daily, with the rationale of twice-daily dosing offering improved protection against DIC. However, painful skin erythema coinciding with a rise in plasma D-dimers reoccurred, indicating insufficient control of coagulation (Figure S2A). Administration intervals of apixaban were shortened to 5 mg every 6 to 8 hours, resulting in sustained DIC control without clinically relevant bleeding

(Figure S2A). Accidental omission of single doses of apixaban, however, prompted an immediate relapse of painful skin erythema, and random blood samples taken during apixaban intake revealed a close inverse correlation between anti-Xa activity and plasma D-dimer levels (Figure S2B).

Over the course of treatment, increasing titers of IgM aCL and β_2-glycoprotein-I antibodies (anti-β_2GPI) were compatible with definite APS (Table 1, Figure 1D,E).[8] Activated protein C (APC) resistance in the absence of *F5* gene mutation, Leiden, indicated an acquired defect in the PC-PS-TM system (Table 1, Table S1). While PS antigen was normal, PS activity was severely reduced. Mixing studies (Figure 2A, Figure S3A,B) and spiking of normal human plasma (NHP) with patient IgM (Figure 2B, Figure S3C) or IgG (Figure 2C, Figure S3D) revealed the presence of an inhibitory IgG autoantibody against PS. In addition, significant microvesicle (MV)-associated tissue factor (TF)-specific procoagulant activity (PCA) was detected in patient plasma (Figure 2D), suggesting that aPL-mediated monocyte activation contributed to DIC evolution
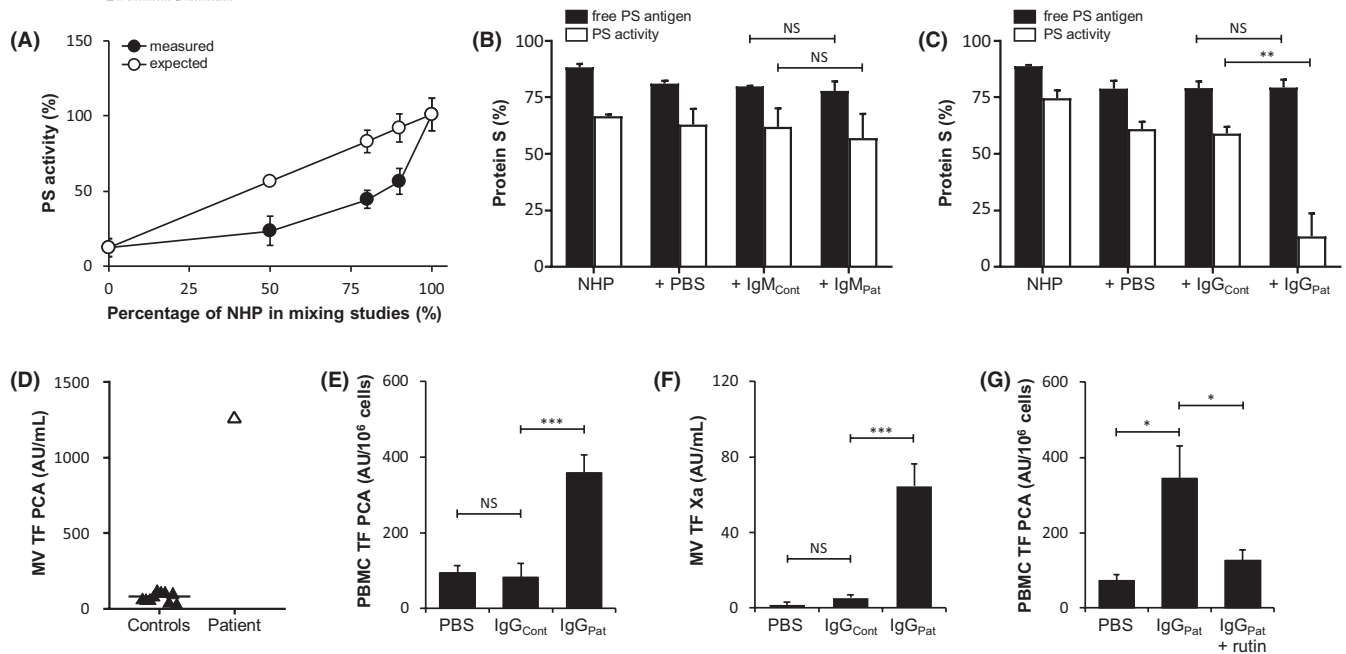
FIGURE 2 Effects of purified patient immunoglobulins on protein S (PS) and peripheral blood mononuclear cells (PBMCs). (A) Patient plasma was mixed with increasing concentrations of normal human plasma (NHP) and subsequently analyzed for free PS antigen and activity (mean ± standard deviation [SD], n = 3). Expected values were calculated on the basis of activity levels measured in 100% patient plasma and NHP, respectively. (B, C) NHP was spiked with phosphate buffered saline (PBS) or 1.5 mg/mL IgM (B) or 7.0 mg/mL IgG (C) from the patient or a sex-matched healthy control. Samples were subsequently analyzed for free PS antigen (filled bars) and PS activity (open bars) (mean ± SD, n = 3). (D) Plasma microvesicles (MVs) were isolated by double high-speed centrifugation from the patient and 10 healthy controls and assessed for tissue factor (TF)-specific procoagulant activity (PCA) by single-stage clotting assay. (E) PBMCs from healthy donors (2×10^6 cells/mL) were incubated with PBS or 500 μg/mL patient or control IgG for 4 h and subsequently analyzed for TF PCA by single-stage clotting assay (mean ± SD, n = 7). (F) Following incubation of PBMCs with PBS or purified IgG, MVs were isolated from culture supernatants and analyzed for TF-dependent Xa generation using a chromogenic two-stage end point assay (mean ± SD, n = 5). (G) PBMCs were incubated with 500 μg/mL patient IgG in the presence or absence of 100 μM of the protein disulfide isomerase (PDI) inhibitor, quercetin-3-ruinoside (rutin), for 4 h and subsequently analyzed for TF PCA by single-stage clotting assay (mean ± SD, n = 4). *p*-values are according to Tukey's post hoc test in panels B and C and E–G (* $P < .05$; ** $P < .01$; *** $P < .001$; NS, not significant)

in the context of PS inhibition. While patient IgM had no effect (Figure S4A), patient IgG specifically amplified TF PCA expression (Figure 2E) and release of TF-bearing MVs (Figure 2F) by peripheral blood mononuclear cells, an effect that was significantly inhibited by the protein disulfide isomerase (PDI) inhibitor rutin (Figure 2G, Figure S4B).

At the time of this report, follow-up of the patient is 6 years, with still no evidence for an underlying rheumatologic, infectious, vascular, or malignant disease. Because intensified oral anticoagulation with apixaban was efficacious and well tolerated, we have so far decided against further immunosuppressive therapy (eg, with rituximab).

3 | DISCUSSION

In our patient, concomitant occurrence of two distinct acquired thrombophilias, APS and autoimmune PS deficiency, induced a severe imbalance of procoagulant and anticoagulant pathways, which resulted in thrombotic DIC and required uninterrupted anticoagulation at (supra)therapeutic dosages.

Previous studies have established PS inhibitors as an independent risk factor for thrombosis. In most cases, PS inhibitors occurred transiently following an infection and were attributed to cross-reacting pathogen-specific antibodies.[1-3,6] In our patient, the inhibitor persisted over the entire observation period of 6 years, indicating an autoimmune rather than a para-infectious phenomenon.

PC activity was also reduced on several occasions (Table 1), and spiking of NHP with patient IgG resulted in a minor decrease in PC activity (Figure S3D). While PC activity in clotting-based assays depends on PS cofactor function, PS is dispensable in chromogenic PC activity assays.[12] Since patient IgG also interfered with chromogenic PC activity (Figure S3D), we cannot exclude the possibility that the patient additionally had an IgG PC inhibitor, contributing to the procoagulant state.

Although PS deficiency predisposes to thrombosis, additional procoagulant stimuli are usually required to trigger clot formation. In secondary APS, coexisting deficiencies in the PC-PS-TM system are associated with an increased thromboembolic risk, but data regarding primary APS are missing.[5,6] In our patient, elevated aPL beyond an otherwise unremarkable autoantibody profile were detected (Figure 1D,E, Table S1). Because aPL persisted over several

years (Figure 1D,E) and immunosuppressive therapy with prednisolone was ineffective, both a transient autoimmune epiphenomenon and secondary APS are highly unlikely.

Primary APS is associated with a continuous state of low-grade inflammation initiated by cofactor-dependent and -independent effects of aPL, including intravascular TF production and release of procoagulant MVs.[11,13,14] Consistent with aPL-mediated monocyte activation, abundant MV-associated TF PCA was detected in patient plasma (Figure 2D). Notably, despite the development of significant IgM aPL titers during follow-up, the capability of inducing TF PCA expression and release of TF-bearing MVs by peripheral blood mononuclear cells (PBMCs) was restricted to patient IgG (Figure 2D-F, Figure S4A). While the pathophysiological relevance of IgM aPL remains controversial,[15] a plethora of additional cofactor-dependent and lipid-reactive aPL, albeit not part of the established diagnostic criteria, have been described.[13,14,16] Such aPL might not have been captured by the applied screening ELISA and routine laboratory tests, but might have contributed to IgG-mediated procoagulant effects.

DIC severity appeared to correlate with aPL titers in our patient. While at initial presentation prophylactic to intermediate dosages of LMWH followed by rivaroxaban 20 mg OD were sufficient to control coagulation activation, 5 mg of apixaban every 6 to 8 hours were required with increasing aPL titers (Figure 1D,E, Figure S2A). Of note, D-dimers significantly correlated with apixaban plasma concentrations (Figure S2B), further indicating a continuous procoagulant state requiring uninterrupted anticoagulation.

While direct oral anticoagulants (DOACs) are safe and efficacious for the treatment and secondary prevention of venous thromboembolism in the general population,[17] their role in thrombotic APS is less clear. In high-risk patients with APS, DOACs might be associated with increased rates of arterial thromboses compared to vitamin K antagonists (VKAs).[18] We have decided against VKAs because of the highly variable dose-response relationship, the unfavorable pharmacokinetic and pharmacodynamic profile, and the potential risk of further impairing the PC-PS-TM system through downregulation of vitamin K–dependent coagulation inhibitors PC and PS. We have previously shown that prophylactic dosages of apixaban improved clinical symptoms and hemostatic parameters in two patients with chronic DIC due to a vascular cause.[19] Further studies are needed to investigate whether intense anticoagulation with apixaban is safe and efficacious in APS with a severe thrombotic phenotype due to a dysfunctional PC-PS-TM system.

Adjunct treatment strategies may be required in patients with APS, in whom anticoagulants (with or without antiplatelet agents) are insufficient to control coagulation activation, with PDI being a promising target. In APS, β_2GPI is under direct oxidoreductive control of PDI,[11] and induction of monocyte TF by cofactor-independent aPL was linked to extracellular PDI activity.[14] Consistently, coincubation with rutin partially reversed the stimulatory effect of patient IgG on PBMC TF PCA (Figure 2G). It is thus tempting to speculate that PDI inhibition represents a promising novel treatment strategy in APS that specifically prevents thrombosis but does not impair hemostasis.

Taken together, autoimmune PS deficiency is a rare but serious complication in primary APS that can lead to a highly prothrombotic state characterized by a detrimental imbalance of procoagulant and anticoagulant pathways. In these patients, intense anticoagulation is required to sufficiently control coagulation.

AUTHOR CONTRIBUTIONS

LB, CCR, and FL designed the study, analyzed data, and wrote the first draft of the manuscript. MV designed experiments, analyzed data, and critically revised the manuscript. KH, ML, SWS, MH, TR, and CB analyzed data and critically revised the manuscript. All authors reviewed the final version of the manuscript and gave approval for its submission.

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