Thrombosis Research 156 (2017) 119–125

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

A novel 2-stage approach that detects complement activation in patients with antiphospholipid antibody syndrome

Jacob H. Rand [a], Xiao-Xuan Wu [a], Lucia R. Wolgast [b], Victor Lei [c], Edward M. Conway [c,*]

[a] Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, New York, NY 10065, United States
[b] Department of Pathology, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY 10461, United States
[c] Centre for Blood Research, Department of Medicine, Faculty of Medicine, University of British Columbia, Vancouver, BC V6T 1Z3, Canada

ARTICLE INFO

Article history:
Received 29 March 2017
Received in revised form 11 May 2017
Accepted 8 June 2017
Available online 9 June 2017

Keywords:
Vascular endothelial cells
Autoantibodies
Phospholipids
Complement activation
Thrombosis

ABSTRACT

Introduction: The antiphospholipid syndrome (APS) is marked by autoantibodies that recognize anionic phospholipids in a cofactor-dependent manner. A role for complement has been implicated in the pathophysiology, however, elevations of complement activation markers have not been consistently demonstrated in clinical studies. We therefore designed a proof-of-principle study to determine whether complement activation might be detectable in APS by first exposing plasmas to phospholipid vesicles.
Methods: We examined complement activation markers in patients with APS, non-APS thrombosis, systemic lupus erythematosus, cancer, patients with antiphospholipid antibodies without thrombosis (APL) and healthy controls. Direct measurements of plasma C5a and sC5b-9 levels were compared to levels that were generated in normal serum by phospholipid vesicles that had been pre-incubated with the same plasmas. We then determined the effects of the C5 inhibitor, eculizumab, examined the complement pathways involved, and determined whether the effects could be reproduced with purified IgGs and β2-glycoprotein I (β2GPI).
Results: Plasma levels of C5a and sC5b-9 were higher, but not significantly increased in APS patients compared to healthy controls. In contrast, phospholipid vesicles pre-incubated with APS plasmas generated significantly higher levels than healthy controls and the other groups, except for APL patients. Complement activation was abrogated by addition of eculizumab. The results with substrate sera indicated that the alternative and classical/lectin pathways were involved. The results were reproducible with purified IgGs and β2GPI.
Conclusion: This proof-of-principle study confirms a role for complement in APS and opens the possibility of monitoring complement activation by including phospholipid vesicles in assay systems.
© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

The antiphospholipid syndrome (APS) is defined by recurrent vascular thrombosis (arterial and venous) and obstetrical complications that are attributable to placental insufficiency, in conjunction with the sustained abnormality of one or more antiphospholipid laboratory assays (lupus anticoagulant, anti-cardiolipin IgG or IgM, anti-beta-2-glycoprotein 1 (β_2GP1) IgG or IgM antibodies) [1]. APS may be isolated or associated with other auto-immune diseases, such as SLE. Although the commonest thrombotic presentation is deep vein thrombosis, patients may rarely present with a life-threatening catastrophic variant, with multi-organ thrombosis [2]. In spite of intense study over the past 3 decades, APS remains an enigmatic and difficult-to-treat disease that is often refractory to currently used anticoagulants [3].

The underlying mechanisms for thrombosis in APS remain poorly understood, but appear to involve dysregulation of the innate and adaptive immune systems [4]. Antiphospholipid antibodies bind directly to a variety of molecules on the surface of anionic phospholipid bilayers of vascular endothelial cells, monocytes, neutrophils, platelets, and syncytiotrophoblasts [5–7]. This results in cellular activation, and an imbalance favoring inflammation and coagulation [8,9], with enhanced tissue factor exposure and inactivation of annexin A5 [10]. Although there are multiple antigenic targets in APS, β_2GP1 is particularly important [9], as revealed in rodent models where administration of anti-β_2GP1 antibodies heightens the risk of arterial thrombosis [11].

There has been increasing recognition of multiple links between complement activation and the activation of the coagulation system (reviewed in [12]). There is also strong evidence implicating excess complement activation in the initiation and progression of APS (reviewed in [13,14]). Several studies have shown that patients with APS have reduced circulating levels of C3 and C4, with increased C3a

Abbreviations: APL, patients with antiphospholipid antibodies without thrombosis; APS, antiphospholipid antibody syndrome; SLE, systemic lupus erythematosus; β_2GP1, beta-2-glycoprotein 1; PS, phosphatidyl serine; PC, phosphatidyl choline; GVB, gelatin veronal buffer; ELISA, enzyme linked immunoassay.

* Corresponding author at: University of British Columbia, Centre for Blood Research, 2350 Health Sciences Mall, LSC 4306, Vancouver, BC V6T 1Z3, Canada.
E-mail address: ed.conway@ubc.ca (E.M. Conway).

http://dx.doi.org/10.1016/j.thromres.2017.06.014
0049-3848/© 2017 Elsevier Ltd. All rights reserved.

and C4a, and variably reported changes in C5a [15–18]. In humans and rodents, deposits of β_2GP1, IgG, C1q, C3, C4 and C5b-9 are specifically found at the sites of vascular injury and fibrin deposition [19,20]. Reduced levels of C3, C4 or C6 confer resistance to antiphospholipid antibody induced thrombosis or fetal loss in small animal models (reviewed in [21]), while pharmacologic blocking of generation of C5a and C5b-9 dampens antiphospholipid antibody mediated thrombosis, fetal loss and tissue factor activity [19,22,23]. Indeed, several patients with the catastrophic form of APS have reportedly responded well to treatment with eculizumab [20,24–26]. Notably, response to treatment does not apparently affect the titer of the antiphospholipid antibodies, whereas elevated markers of complement activation are reduced [20,27].

Despite these insights, and as noted above, systemic activation of complement is not consistently detected in patients with APS, suggesting that the sensitivities and specificities of current assays may be inadequate [17,18]. This may be due to the fact that complement activation is triggered locally at sites of injury, and circulating activation products (e.g. C3a, C4a, C5a, sC5b-9) become diluted in the blood and/or are rapidly cleared.

The idea of including synthetic anionic phospholipid vesicles in assay systems is not completely novel. Phospholipids are included in a variety of coagulation assays, where they are essential for the assembly of coagulation enzyme-substrate complexes, and for lupus anticoagulant assays. There they are targeted by aPL antibodies and cofactors that interfere with phospholipid-dependent coagulation reactions [28]. Since thrombosis is dependent on anionic phospholipid surfaces, and complement and coagulation co-amplify, we considered it reasonable that these vesicles might be similarly useful in facilitating the detection of plasma-derived APS complexes on complement activation.

We therefore investigated whether synthetic anionic phospholipid vesicles, representing the cellular injury site, can be used to concentrate patient plasma-derived triggers of complement activation, thereby providing a more sensitive means of detection. By this technique, we show, in these proof-of-principle studies of well-defined groups of patients, that APS patient plasma constituents that bind to anionic phospholipids, specifically induce complement activation via the classical/lectin and alternative pathways.

2. Materials and methods

2.1. Preparation of phospholipid vesicles

Phospholipid vesicles (30% phosphatidyl serine (PS) and 70% phosphatidyl choline (PC)) were prepared as reported [10], and were suspended in HEPES buffered saline (HBS) at 5 mM.

2.2. Ethics

The institutional review board of Montefiore Medical Center granted permission for the use of anonymized excess human plasmas from clinical assays, and the Weill Cornell Medical College review board deemed anonymized and discarded blood bank plasmas to have exempt status; all of the plasmas were stored at −70 °C. Approval for working with human plasmas was obtained from the University of British Columbia Research Ethics Board.

2.3. Patient groups (Table 1)

Citrated plasma was obtained from patients with well-defined APS with a history of venous thromboembolism (n = 19) (mean age 37.7 yrs) [1]. The spectrum of consensus-based antiphospholipid assays is shown in Table 2. All of the APS patients were lupus anticoagulant (LA)-positive; 7 of these were positive for LA alone while the remainder had elevated levels of various combinations of anticardiolipin and anti-β2-glycoprotein I (β2GPI) antibodies. Additionally, all but 5 of those patients were known to have been treated with anticoagulants; the treatment status was unknown for 4 of the patients, and one was treated with hydroxychloroquine alone. Plasma was also obtained from patients with a history of thrombosis, but without APS (VTE) (n = 17) (mean age 49 yrs), patients with SLE lacking a history of thrombosis

Table 1
Demographic information.

Sample	APS			Cancer alone		SLE alone		Non-APS VTE			APL	
Number	Age	Sex	Thrombosis	Age	Sex	Age	Sex	Age	Sex	Thrombosis	Age	Sex
1	21	F	DVT	67	M	29	F	67	M	DVT, PE	19	F
2	49	F	DVT, PE	68	M	18	M	27	F	DVT, PE	57	M
3	25	F	DVT	73	M	49	F	24	F	SST	50	M
4	45	F	DVT	70	M	34	F	55	M	DVT, PE	34	M
5	22	M	DVT, PE	46	F	19	F	90	F	DVT	20	M
6	50	M	DVT, PE	81	F	35	M	67	F	DVT	38	F
7	21	M	DVT	62	F	61	F	50	F	DVT, PE	49	F
8	24	M	RVT	67	F	36	F	28	F	DVT	46	M
9	43	M	Stroke	57	F	56	F	55	M	DVT, PE	38	F
10	54	F	DVT	75	F	20	F	21	F	DVT, PE	28	F
11	46	F	Stroke	86	M	29	F	51	M	DVT	69	M
12	21	F	TIAs	58	F	32	F	55	M	PE	30	F
13	50	M	DVT, PE	48	M	39	M	35	M	DVT	62	F
14	23	F	DVT	61	M	50	M	78	M	PE	39	F
15	38	M	DVT, PE	84	F	54	M	70	M	DVT, PE	65	F
16	43	M	Stroke	69	F	23	F	33	M	DVT, PE	35	F
17	55	F	DVT	83	M	53	F	27	F	DVT	53	F
18	64	M	DVT	74	M	43	F				38	M
19	22	M	DVT, PE	53	F	61	F				18	M
20				34	F	47	M				42	F
Mean	37.7			65.8		41.5		49			41.5	
SD	14.5			13.7		14.9		21			14.9	

Citrated plasmas were obtained from 5 groups of patients (n = 17–20 for each group):
- All plasmas from patients with APS (i.e., with thrombosis) and APL (without thrombosis) had positive LA, and with/without positive anticardiolipin IgG/IgM/IgA or antiphospholipid IgG/IgM or anti-ß2GPI IgA/IgG/IgM antibodies.
- All plasmas from patients with cancer, non-APS-associated thrombosis (VTE), and SLE without thrombosis, had negative LA, and negative antiphospholipid immunoassays.
- Control citrated plasmas (n = 15) were obtained from healthy volunteer anonymized blood bank donors.

(Thrombosis: DVT = deep vein thrombosis; PE = pulmonary embolus; RVT = retinal vein thrombosis; SST = sagittal sinus thrombosis; TIAs = transient ischemic attacks; M = male. F = female).

Download English Version:

https://daneshyari.com/en/article/5621876

Download Persian Version:

https://daneshyari.com/article/5621876

Daneshyari.com