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Short communication

A potential anti-coagulant role of complement factor H

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ABSTRACT

Anti-phospholipid syndrome (APS) is a complex autoimmune disease, associated with recurrent venous and arterial thrombosis in various tissues. APS is associated with specific antibodies against plasma beta-2 glycoprotein 1 (β2-GP1), and these antibodies react with β2-GP1 bound to negatively charged phospholipids (e.g. cardiolipin) on cell membranes. Some APS patients also have autoantibodies to complement factor H (FH), a homologue of β2-GP1, which also binds to anionic phospholipids. β2-GP1 has earlier been shown to inhibit the intrinsic (contact) activated blood coagulation pathway, promoted by anionic phospholipids. Here we examine whether FH could have similar anti-thrombotic properties. In vitro experiments with surface-bound phospholipids and human plasma, in the presence of FH, confirm this hitherto unreported property of FH.

1. Introduction

Anti-phospholipid syndrome (APS), also called Hughes syndrome, is a heterogeneous, yet distinct autoimmune disease, which is characterised by recurrent systemic thrombosis, appearing as deep venous thrombosis, pulmonary embolism, or as arterial cerebral thrombosis associated with ischemic stroke and myelitis, and by thrombocytopenia. In pregnancies there are also recurrent spontaneous abortions (Hughes, 1983; de Laat et al., 2009a). APS can occur as a primary disease, or in association with other diseases such as systemic lupus erythematosus (SLE). As first defined, APS appeared to be mediated by autoantibodies against negatively charged phospholipids and sulfatides, including cardiolipin (CL), phosphatidylserine (PS), and by lupus anticoagulant (LA) antibodies (Hughes, 1983; Miyakis et al., 2006). However, the pathogenic anti-phospholipid autoantibodies (aPL) have been found to be mainly directed against proteins such as β2-GP1, but only when these proteins are bound to cell membrane or solid-phase anionic procoagulant phospholipids (Schousboe, 1985; McNeil et al., 1990; Guerin et al., 1997; Arvieux et al., 1999; Tripodi et al., 2011). The aPL targets may include coagulation proteins, vascular endothelial cells and platelets, normally protected from exerting a thrombotic effect by β2-GP1 (Vazquez-Mellado et al., 1994; de Groot and Urbanus, 2012a). The anionic phospholipids, exposed due to injury or infection, may trigger in genetically susceptible people an APS episode, by activating the FXII (Hageman) contact phase of the intrinsic blood coagulation pathway (Schousboe, 1985; Rojkjaer and Schousboe, 1997). Extrinsic tissue factor TF/FVII complex and the common blood-coagulation pathway may also become involved (Nimpf et al., 1986). Unlike the APS autoantibodies directed against the cardiolipin/cofactor complex, anti-cardiolipin antibodies, which occur in syphilis, may directly bind to phospholipids. These antibodies are not considered to predispose to APS (Hughes, 1983; Matsuura et al., 1990; de Groot et al., 2012b). In normal people, i.e. those without an increased risk for the disease, as estimated by its lack of history and of persistence of pathogenic autoantibodies such as of LA, anti-cardiolipin and of anti-β2GP1 (Amaral et al., 2010), natural autoantibodies with a low affinity for β2-GP1 have been detected, apparently of unclear function (de Groot and Urbanus, 2012a).

FH is a homologue of β2-GP1 which prompted us to investigate common properties. We detected specific IgG autoantibodies against FH in a number of APS patients, suggesting their contributory role in this thrombophilia (Guerin et al., 1997, 1998; Ferluga et al., 1998). A FH anti-thrombotic role in APS would be consistent with observations on the relative deficiency of this fully functional regulator associated with the atypical haemolytic uremic syndrome (aHUS). aHUS is characterised by complement dysregulation and

Abbreviations: APS, anti-phospholipid syndrome; β2-GP1, beta 2-glycoprotein 1; FH, factor H; SLE, systemic lupus erythematosus; CCP, complement control protein; CL, cardiolipin; PS, phosphatidylserine; aPL, anti-phospholipid autoantibodies.

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thrombotic microangiopathy (Rodriguez de Cordoba et al., 2004; Licht et al., 2005; Hakobyan et al., 2010; Meri, 2013). Autoantibodies to FH have been found in several diseases, including aHUS and SLE, and confirmed in thrombotic patients having also LA antibodies, a marker for APS (Guerin et al., 1998; Meri, 2013; Zadura et al., 2012).

β2-GP1 (Apolipoprotein H) is an abundant plasma protein, partially associated with lipoprotein fractions, and is composed of 5 complement control protein (CCP) modules/domains (Steinkasserer et al., 1991). β2-GP1 does not possess the complement regulatory activities of FH (i.e. decay-acceleration and factor I-cofactor activity (Puurunen et al., 1995).

However, a complement regulatory role for β2-GP1, connected with that of FH, has been suggested recently. Gropp et al. (2011) reported that β2-GP1, when bound to an anionic phospholipid surface, can bind unactivated C3, and make it susceptible to the action of FH and factor I.

Previously, β2-GP1 was found to inhibit the anionic phospholipid contact activation of blood coagulation (Schousboe, 1985), platelet prothrombinase activity (Nimpf et al., 1986; Vazquez-Mellado et al., 1994), and ADP induced platelet aggregation (Nimpf et al., 1987). In 90% of the population, β2-GP1 is found at 150–300 μg/ml in plasma (Schousboe, 1988). β2-GP1 circulates as a closed, seemingly inert form (de Groot and Urbanus, 2012a). However, it acquires a stretched conformation upon binding *via* its CCP5 C-terminus domain containing the highly positive amino acid –KNKEKK– motif, binding to anionic phospholipids (Kertesz et al., 1995; Steinkasserer et al., 1991). It may thus expose the hidden antigenic epitopes located on the N-terminus in the first CCP module, so as to interact with pathogenic antibodies (Musial, 2012; de Laat et al., 2009a). Several heterogeneous β2-GP1 conformational epitopes in the first CCP region are recognised specifically by the APS antibodies (Guerin et al., 1997, 2000). According to a pathogenesis model, the antibody/β2-GP1 complex may be taken up by a range of cellular receptors, upon removing β2-GP1 from anionic phospholipids, thereby activating endothelial cells and platelets, and exposing the phospholipids for thrombus formation (Tripodi et al., 2011; de Groot and Urbanus, 2012a).

Complement FH (155 kDa) is a versatile plasma glycoprotein of variable concentration range with mean concentration around 220–240 μg/ml. FH is made of 20 CCP modules and is encoded within the chromosome 1q32 cluster of Regulators of Complement Activation (RCA) genes, together with membrane bound regulators such as the decay accelerating factor (DAF), CR1, CR2, the membrane cofactor protein (MCP), C4 binding protein (C4 bp), and FH related proteins FHR 1-5. FH has an alternatively-spliced form, FHL-1, which consists of the first 7 CCPs of FH followed by the unique tetrapeptide sequence SFTL (Ripoche et al., 1988). For their alternative pathway regulation, FH and some of these other RCA cluster proteins share C3bBb decay accelerant property, by dissociating C3 and C5 convertases C3bBb and $C3b_2Bb$, through binding to C3b (Ripoche et al., 1988). When bound to cell surfaces, FH protects cell and tissues from auto-complement attack. The CCP18-20 and CCP 6–7 modules of FH bind to cell polyanions such as glycosaminoglycans (GAGs), enhancing its binding to C3b, already covalently bound to the membrane (Clark et al., 2006).

In addition to β2-GP1, FH as well as CR1 and C4 bp, have been found to be distinct targets of autoantibodies associated with APS. As in the case of β2-GP1, the FH antigenic epitopes were recognised by distinct autoantibodies only when bound to a solid surface, possibly exposing the specific pathogenic epitopes. β2-GP1 and FH competed for binding to anionic phospholipids (Kertesz et al., 1995; Guerin et al., 1998; Ferluga et al., 1998; Tan et al., 2010). Thrombotic patient antibodies recognised C4 bp epitopes when in combination with CL (Arvieux et al., 1999). C4 bp is involved in coagulation regulation, by complexing protein S, a cofactor of anti-coagulant activated protein C (Guerin et al., 1998; Dahlback, 2011).

Here, we report that FH, like β2-GP1, also inhibits the anionic phospholipid activated Hageman factor XII contact coagulation pathway.

2. Materials and methods

2.1. Proteins

FH was prepared as indicated in study by Sim et al. (1993). β2-GP1 was prepared as described by Williams and Sim (1993). α_2M was purified as described previously (Harpel, 1976). CL, PS, PA and APTT were purchased from Sigma Aldrich. The activated partial thromboplastin time (APTT) coagulation test reagent contains rabbit brain cephalin dissolved in buffered 0.1 mM ellagic acid. The chromogenic substrate H–D-Pro-Phe-Arg-pNA (S-2302™) was from Chromogenix AB. Human IgG was made by triple Na_2SO_4 precipitation from human serum (Johnstone and Thorpe, 1996).

2.2. Amidolytic reaction and tests

The previously published procedures for β2-GP1 inhibition testing of Hageman factor system, activated on contact with phosholipids in human plasma, were adapted (Schousboe, 1985, 1988). Kallikrein amidolytic activity generated in human phospholipid coated microtitre plates, was measured by the chromogenic substrate S-2302™ hydrolysis. The rate of formation of the cleaved yellow product, *p*-nitroaniline (pNA) was read at 405 nm.

To obtain human plasma, blood was drawn with a syringe, containing 25 mM EDTA at pH 7.5, to a dilution of about 2.5 mM EDTA. After centrifugation at 10,000 *g* for 10 min, the plasma was aliquoted into small tubes and stored at −70 °C.

Microtitre plates (Falcon, flexible and of low adsorbance capacity, with slightly narrowing conical flat bottom wells) were coated with 50 μl/well of 50 μg/ml negatively charged phospholipid ethanol solution, and the plates dried overnight in an air stream. In the tests, 50 mM Tris–HCl, 12 mM NaCl buffer, pH 7.8 was used (assay buffer). The indicated amounts of FH and of other proteins, dialysed against the above buffer, were added to the wells and supplemented with the buffer up to 55 μl volume, and incubated at 37 °C for 20–40 min. Then 20 μl of plasma diluted 1 to 10 with the assay buffer was added, and the mixture incubated for 2–3 min at 37 °C, followed by the addition of 15–20 μl of 1/5 assay buffer diluted 1.2 mM S-2302. The amidolytic reaction was stopped after 2–3 min with the addition of 20 μl 25% acetic acid, and the *p*-nitroaniline absorbance measured. In some cases the amidolytic reaction was continued and read in a few one minute intervals. As a control, assay buffer and the S-2302 were added to untreated wells. The background amidolysis level was found to be almost equal, or slightly higher, to that with the neutral phosphotidycholine (PC) coated wells shown in Fig. 2.

The APTT test reagent was also examined for its potential inhibition by FH. The commercial APTT solution, diluted 1/10 in assay buffer, was added in 10–20 μl volumes to the non-coated wells together with the examined proteins, the volume adjusted with assay buffer, incubated at 37 °C, and the test continued as above. Preliminary tests indicated an APTT concentration balance against FH inhibition of contact activation (not shown).

Studies with purified Hageman components, and with whole human plasma, at physiological ionic strength, indicated a requirement for Zinc $^{2+}$ ions and of HMW kininogen, for optimal contact activation by anionic inositol-phospholipid vesicles. Ca^{2+} ions are not needed in Hageman factor phase (Schousboe, 1990).