Association of anticardiolipin antibodies with vascular injury: possible mechanisms

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Lupus anticoagulant, first observed in 1952 in two patients with systemic lupus erythematosus (SLE),¹ was linked to a phospholipid antigen when positive Wassermann tests for syphilis coincided with the lupus anticoagulant, documenting the lipid nature of the antigen. Bowie and colleagues were the first to unravel the paradoxical prothrombotic tendency of patients with positive lupus anticoagulant.² This prothrombotic tendency that often results in microscopic thromboembolism is unique in being detected by positive serological tests. While immunoglobulins of isotypes IgG or IgM may have the lupus anticoagulant effect, the significance of IgA isotypes is not clear. These antibodies interfere with the *in vitro* phospholipid dependent coagulation tests. Lupus anticoagulant activity is documented by inability to correct prolonged activated partial thromboplastin time (aPTT) with normal plasma, and the necessity of phospholipid for the inhibition. Lupus anticoagulant belongs to a family of antiphospholipid antibodies (aPL) that also includes reagin and anticardiolipin antibodies (aCL), and the recently recognised antibody to beta-2-glycoprotein I (β 2GpI). aCL and lupus anticoagulant are distinct and complementary tests for aPL. In patients with aPL syndrome, 60% will be positive for both lupus anticoagulant and aCL, while either test will be positive in the rest of the patients.

Until recently, the most sensitive test for aPL was aCL. This ELISA test determines antibody binding to solid plates coated with cardiolipin or other negatively charged phospholipids. Recently, aPL were shown to be specific for phospholipid-protein complexes,³ where the protein is identical with being β 2GpI,^{4,5} or less often prothrombin, protein C, or protein S.⁶⁻⁸

aPL may be either autoimmune or alloimmune. The latter appear transiently after an infection or associated with a lymphoproliferative disorder, and do not usually have clinical implications.

The former are persistent and may be primary, as in the aPL syndrome, or secondary to drugs, SLE (where 40% of patients have either lupus anticoagulant or aCL), or other collagen vascular disease. Autoimmune aPL rather than alloimmune, are associated with hypercoagulability. Although in general thrombotic episodes may occur anywhere in the vascular tree, in a single patient the event is either venous or arterial and does not cross react. Clinical manifestations of aPL include venous and arterial thromboembolic phenomena, such as miscarriage, thrombocytopenia, neurological manifestations, deep vein thromboses and pulmonary embolism, or arterial thromboses. Pre-eclampsia has also been reported to be

associated with aPL. The syndrome may be either a part of a systemic disorder, classically SLE, or a primary disorder, called the antiphospholipid syndrome. While aPL are in fact a family of antibodies, the incidental finding of positive serology is yet to be determined. However, correlation of incidental aPL with thrombotic episodes has not been documented in large scale surveys. After the discovery of aPL, several mechanisms have been proposed for the pathogenesis of its prothrombotic tendency, such as reduced inhibition of activated factor V,⁶⁻⁸ platelet activation,^{9,10} endothelial injury,¹¹ and direct recognition of phospholipids. However, the probable primary mechanism of aPL hypercoagulability has recently been elucidated. β 2GpI is a 50 kD protein with a natural anticoagulant activity. It inhibits surface mediated activation of prekallikrein and factor XII, activated factor V, and platelet aggregation.¹²⁻¹⁴ β 2GpI binds to phospholipid and optimises the reaction in autoimmune diseases but not after infections.¹³ aCL activity is dependent on the combination of cardiolipin and β 2GpI, with the phospholipid binding protein complex playing the major immunogenic part in the production of aCL specifically and the aPL syndrome in general.^{15,16}

Moreover, β 2GpI can discriminate between autoimmune aCL and alloimmune aCL,¹⁷ by enhancing the cardiolipin binding in aPL syndrome and inhibiting the cardiolipin binding of post-infectious aCL. These non-pathogenic alloimmune aPL may react with solid phase and fluid phase cardiolipin even in the absence of β 2GpI, unlike autoimmune aCL.¹⁸

The prevalence of aCL in arbitrary internal medicine patients is 11%,¹⁹ a rate similar to aCL found in young adults with premature atherosclerosis.²⁰ However, 26% of a general population of vascular surgery patients are aCL positive.²¹ Higher prevalence of aCL may also be found in patients with recurrent vascular insults, such as dialysis patients.²²⁻²⁶ In contrast, the proportion of positive aCL in the general population is below 10%.¹⁹

The mechanisms involved in the association between aCL and vascular disease may include one or any combination of endothelial activation, atherogenic, immune, or apoptotic processes in genetically susceptible subjects.

Endothelial activation

aCL have been documented in subendothelial cardiac deposits,²⁷ while histological findings in aCL associated brain infarcts are non-inflammatory and consist of endothelial hyperplasia and thrombosis.²⁸ The mechanism of aCL associated vasculopathy includes

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interaction of endothelial cells with platelets and aPL, to promote a cascade of reactions yielding recurrent local thromboses and intimal hyperplasia. Anticardiolipin autoantibodies prompt a prothrombotic endothelial surface,²⁹ while the β 2GpI-aCL antibody complex activates endothelium in vitro.³⁰

Antiphospholipid antibody binding to endothelium induces in vitro up-regulation of adhesion molecules, such as intracellular adhesion molecule-1 and extracellular adhesion molecule-1, stimulated by an autocrine loop of interleukin (IL)-1 β secretion.³¹

Platelet-endothelium interaction mediated by aCL may alter thromboxane A2-prostacyclin balance, leading to enhanced thrombosis and vasoconstriction.³² Patients with renal failure may be especially prone to this effect because uraemia is associated with the inhibition of nitric oxide synthase,³³ and because of the effect of hypertension and advanced glycosylation end products on endothelial cell relaxation and proliferation, and on endothelin production in vitro.^{34, 35}

Endothelin-1 which induces vasospasm and arterial occlusion, is released by endothelium in response to aPL.³⁶ The molecular mechanism may involve endothelial adhesion via β 2GpI lysine 286, and activation of endothelial cells by the complex of aCL and anti- β 2GpI,³⁷ resulting in inhibition of endothelial antithrombotic effect and vascular tone relaxation. A mechanism similar to heparin induced thrombocytopenia has also been suggested for aCL associated vascular occlusion and thrombosis.³⁸

In addition to endothelial induced thrombosis, intimal hyperplasia plays a major part in vascular occlusions. Homocysteine induces endothelial modulated vasoconstriction and growth of vascular smooth muscle cells,³⁹ and endothelin, prompted by aCL, enhances endothelial cell proliferation in vitro.³⁴

Vascular endothelial growth factor (VEGF), also named vascular permeability factor (VPF), may be an important regulator of angiogenesis in inflammation.⁴⁰ VEGF/VPF may be extensively expressed in wall or plaques of medium to large vessels and in narrowed atherosclerotic arteries.⁴¹ However, the precise pathogenic role of VEGF in vascular diseases is yet to be determined.

Initial endothelial damage exposes anionic phospholipids that react with phospholipid binding proteins, such as β 2GpI or prothrombin. The simultaneous binding of aCL to cellular Fc receptor and phospholipid protein complex induces endothelial-platelet interaction resulting in thrombosis.

Accelerated atherosclerosis

An intriguing possible pathogenetic role for aCL in vasculopathy is the cross reaction with oxidised low density lipoprotein (LDL) antibodies. Oxidised LDL may induce IL-1 secretion by macrophages, resulting in smooth muscle proliferation. Oxidative modification of LDL plays a major part in atherogenesis via foam cell formation and cell cytotoxicity, with uptake of oxidised LDL-antioxidised LDL complexes by macrophages. As phospholipids

bear structural resemblance to LDL, aCL may cross react with antioxidantised LDL.⁴²

On the one hand, oxidised LDL aggravates in vitro the clinical manifestations of aPL,⁴³ and on the other hand, atherogenic effect of human lupus sera in vitro may be mediated by LDL-containing immune complex.⁴⁴ Each cardiolipin molecule contains four unsaturated fatty acids, highly susceptible to oxidation. Mice sera with high titres of oxidised LDL antibodies, bind cardiolipin effectively only after oxidation. Therefore oxidative events may also play a major part in aCL formation.⁴⁵ Because aCL may cross react with oxidised LDL antibodies, which recognise atherosclerotic lesions and not normal arteries, their association may not be incidental in the atherosclerosis-prone dialysis patients. Indeed, in patients prone to arterial thrombosis, antioxidantised LDL antibodies may be markers of antiphospholipid syndrome, and levels of antioxidantised LDL antibodies are higher in these patients.⁴⁶

LDL may also be involved in aCL associated vascular occlusion by inducing a prothrombotic state.

LDL itself may be a thrombogenic target of aCL,⁴⁷ and raised concentrations of lipoprotein (a) in patients with aPL may inhibit the fibrinolytic pathway.⁴⁸ Interestingly, in patients with SLE, 10% of aCL positive patients had a myocardial infarction.⁴⁹

Apoptosis

Hypercoagulability in aPL may also be induced by apoptotic processes. Alterations of the phospholipid phase of cell membranes during late apoptosis are immunogenic and associated with the production of aPL. These surface alterations also have an independent procoagulant activity.⁵⁰ Apoptotic cells may promote coagulation directly or via atherosclerotic plaque dislodgment.

Characteristic membrane blebbing, occurring in final stages of apoptosis may lead to the production of aPL and tissue factor procoagulant activity. Moreover, it has been claimed that via this pathway aPL may exert their hypercoagulability.⁵⁰ Apoptotic inflammatory cells, such as macrophages and T cells, are found abundantly in atherosclerotic plaques and may induce plaque instability. Endothelial cell apoptosis may lead to loss of anticoagulant activity and increased leucocyte and platelet adhesion, resulting in rapid progression of the atherosclerotic and calcification process.⁵⁰ On the other hand, aPL may enhance apoptosis, with nuclear DNA fragmentation, cell lysis, and membrane disruption.

Autoimmunity

Pathogenicity of aPL depends on specificity, isotype, level, and duration of aCL.⁵¹ Cross regulatory roles of immunity and autoimmunity have recently emerged in vasculopathic and atherosclerotic processes.⁵² A prerequisite for this vascular autoimmunity is a humoral or cellular immune reactivity to self antigens,⁵³ such as LDL turned immunogenic during oxidation and glycation and prompting T cell mediated immunity.⁵⁴⁻⁵⁷

Anti- β 2GpI antibodies are of low affinity, supporting the need for β 2GpI-phospholipid interaction. In asymptomatic cases, the aCL is usually of low titre and of IgM isotype, while autoimmune aCL are predominantly IgG, mainly IgG₂ and IgG₄, and high titre.

aPL IgM is not routinely considered significant, unless uniquely and persistently documented in hypercoagulable states.

Genetic predisposition

Most aCL are species specific recognising only human plasma. aCL are associated with class II major histocompatibility complex and in particular the DQB1 locus⁵⁸ and DRw53, and either DR4 (in Caucasians) or DR7 (in Latinos).⁵⁹ Genetic factors probably play an important part in the thrombogenic mechanism of aPL.²⁷ Therefore, aCL may occur in genetically or immunologically susceptible haemodialysis patients, after a common infection or after recurrent endothelial insults and local thromboses.

aCL and anti- β 2GpI antibody titre are usually well correlated.^{60–61} The underlying explanation may be the varying affinities of aPL family to protein-phospholipid complex. Excluding β 2GpI, aCL may be directed against the complex of phospholipid and prothrombin, protein C, and protein S.³⁸

Therapeutic options for aCL associated vasculopathy

Common practice mandates treatment only for aCL associated thrombosis and not for incidental finding of aCL. Possible options include oral anticoagulants with an ideal international normalised ratio of 3.5, or aspirin and subcutaneous heparin. Glucocorticoids were beneficial in a few patients with aPL associated valvulitis.⁶² New therapeutic strategies may also involve antioxidant agents in haemodialysis associated vascular events.⁴⁵ Future prospects may include genetic modification of antithrombogenic nature of endothelial blood interface in vascular grafts by specific recombinant DNA insertion into seeded endothelial cells.⁶³ A better understanding of the functional balance between positive and negative effects on endothelial cell proliferation may pave the way to new therapeutic prospects.

Conclusions

IgG aCL indicate chronic immunity, representing either a reaction to a primary endothelial injury,²⁸ or playing a primary role in vascular occlusive disease.⁶³ Vessel injury may determine the site of vascular thrombosis.²⁰ Therefore, in patients with vascular disease a possible scenario may involve endothelial injury and membrane perturbation exposing neoantigens and adhesion molecules,^{64–65} and the binding of aPL. An underlying genetic predisposition may be the link between recurrent endothelial insults and endogenous immune reaction in susceptible haemodialysis patients. Additionally, both oxidised LDL and the complex of altered β 2GpI epitope and negatively charged phospholipids may be the targets of aCL, prompting the phospholipid dependent primary thrombosis.⁶² Thus, convergence of atherogenic risk factors and aCL associated thrombophilia may play an important part in vascular graft premature occlusion. However, on the other hand, aCL may be merely markers of vascular injury.¹⁴

It would be prudent therefore to conclude that whether aCL are endothelial platelet activators in vascular occlusions, or only play the part of “bystanders”, is not currently known. Testing for aCL is essential for unexplained vascular occlusion. A prospective clinical study will better define the pathogenic role of aCL in the natural history of vascular occlusion disorders.

This review is summarised in table 1.

Table 1 Mechanisms possibly mediating anticardiolipin antibody associated vascular injury

Mechanism	Pathophysiology	References
Endothelial activation	Platelet-endothelial interaction	27–29
	Intimal hyperplasia	28
	Up-regulation of adhesion molecules	31
	Alteration of thromboxane A2-prostacyclin balance	32
Accelerated atherosclerosis	Endothelin-1	36
	Cross reaction with oxidised LDL antibodies	43, 46
	IL-1 secretion by macrophages	41
	Smooth muscle cells proliferation	41
Apoptosis	Thrombogenic effect of LDL and lipoprotein (a)	43
	Membrane blebbing	30
	Apoptotic macrophages and T cells	30
	Endothelial cell apoptosis	30
Autoimmunity	Humoral and cellular immunity for self antigens	53
	Immune recognition of LDL after oxidation and glycation	54
	T cell mediated immunity	56, 57
Genetic predisposition	DQB1 locus, DRw53 locus	58
	DR4 in Caucasians and DR7 in Latinos	59

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