A toggle switch linking coagulation and innate immunity in antiphospholipid antibody syndrome

Title:

A toggle switch linking coagulation and innate signalling is turned on in antiphospholipid antibody-dependent thrombosis and autoimmunity

Antiphospholipid antibodies recognize a lipid-protein receptor complex that is a toggle switch linking coagulation and innate signalling and perpetuates a self-amplifying autoimmune signalling loop

The two sides of some coin: antiphospholipid antibodies triggering immune dysregulation and thrombosis

A single cell-surface lipid–protein receptor complex: new piece in the mosaic of autoimmunity and coagulation

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Abstract:

The recognition of a cell surface antigenic complex constituted by the endothelial protein C receptor and the endosomal lysobiphosphatidic acid as a target for antiphospholipid antibodies (aPL) might represent a crossroad between immune dysregulation, antimicrobial host defense and hemostasis. Recent evidence from in vitro and animal models showed that blocking this target can reduce the pro-thrombotic effect of aPL as well as mitigate the self-amplifying autoimmune pathways. The paradigm of the current understating of antiphospholipid syndrome centered on the pathogenic role of anti-β2-glycoprotein 1 antibodies is overturned by these new evidences. If clinically confirmed on a clinical ground, the identification a single cell-surface lipid–protein receptor complex might support the rational for therapies beyond anti-thrombotic approaches.

Antiphospholipid syndrome (APS) is an autoimmune disease characterized by the presence of antiphospholipid antibodies (aPL), such as lupus anticoagulant, anticardiolipin antibodies and anti-β2-glycoprotein 1 antibodies. APS can present with a variety of clinical phenotypes, with thrombotic manifestations potentially occurring in any vessels, arterial or venous, large or microvasculature, as well as pregnancy morbidity. The pathophysiological hallmark is thrombosis, but other factors such as complement activation might be important (1)

A recently published study Muller-Calleja et al (2.Science) demonstrated that aPLs recognize a single cell surface lipid-protein receptor complex and perpetuates a self-maintaining and amplifying signalling loop dependent on the cooperation with the innate immune complement and coagulation pathways.

Identification of the mechanisms involved in aPL signalling that elicit the devastating effects of the aPL-related syndrome is incomplete. Unless Beta 2GPI-reactive aPL signalling, lipid-reactive aPLs induces translocation of toll-like receptors 7 and 8 (TLR 7 and 8) to endosome promoting endosomal proinflammatory signalling of extracellular RNA in monocytes (12). Based on Muller-Calleja's study this mechanism is unique to lipid reactive aPLs. As shown in this study, IgG isolated from patients with APS showed three distinct reactivities towards cardiolipin alone, cardiolipin plus Beta2GPI, and Beta2GPI alone. The latter was found to be present in restricted minority of cases (10%) and did not induce substantial pro-inflammatory effects in mouse monocytes and trophoblast cells. Conversely, IgG reactive to lipid either alone or cross-reactive with Beta2GPI (cumulatively 90% of cases) induced TNF mRNA expression in both cell types. Accordingly, the human monoclonal aPL HL5B reactive with cardiolipin (but not HL7G reacting with Beta2GPI (5)) inhibited the activation of protein C on endothelial cell. As known, the activated protein C plays an important role in regulating anticoagulation and maintaining the permeability of blood vessel walls.

Lipid reactive aPLs also up-regulates the coagulation initiator tissue factor (TF) which is central to pregnancy morbidity and the prothrombotic monocyte activation, and initiates coagulation in combination with its ligand, namely the protease coagulation factor FVIIa. In quiescent monocytes TF is inhibited by the TF pathway inhibitor (TFPI). Lipid reactive aPLs are able to disrupt the complex TF/TFPI. TF binds to FVIIa and produces the TF-FVIIa-generated factor Xa, FXa. FXa engages the endothelial protein C receptor, EPCR (that physiologically has vascular cyto-protective functions) and cleaves the protease-activated

receptor 2, PAR2 (18REF), which ultimately promotes interferon production by dendritic cells.

Muller-Calleja's study (2.Science) shows that EPCR serves as cell surface receptor and mediates aPLs and EPCR internalization. This effect implies the exchange of EPCR-structurally bound phosphatidylcholine with a late endosomal lipid (i.e., lipid that is not detected elsewhere in the cell, including in early endosomes), the lysobisphosphatidic acis (LBPA). Intact IgG-aPL, able to activate complement, is a prerequisite since F(ab')2 fragment, albeit able to bind cell surface, is not internalized and does not mediate EPCR internalization. The same negative effect can be achieved by adding a complement inhibitor. While binding to cell surface EPCR-LBPA, aPLs disrupt the inhibited TF complex and activate coagulation, but also promote translocation of acid sphingomyelinase to cell surface where it is activated by EPCR-LBPA and turns monocyte membrane to a pro-coagulant phenotype. Incidentally, the same is observed in embryonic trophoblast cells. Moreover, EPCR-LBPA engagement induces the production of alfa-interferon by dendritic cells that eventually sustains a TLR 7-dependent expansion of B1a cells and enhances production of aPLs.

All in all, these observations add furthers pieces in the mosaic of autoimmunity and coagulation involving aPL. In 2011, Prinz et al. [28REF] demonstrated increased expression of TLR7/8 by peripheral blood mononuclear cells (PBMCs) in the specific population of APS patients. In this study exposing **p**DCs to either total IgG fractions from patients or non-cofactor-dependent monoclonal aPL promoted IFN-α expression in a TLR7-dependent manner. This only occurred in the presence of traditional TLR7 ligands such as single-stranded RNA, suggesting that aPL sensitize pDCs to these ligands [28REF]. Mechanistically, aPL appeared to prime pDCs to internalize RNA, while also promoting translocation of TLRs from the endoplasmic reticulum to the endosome [28REF]. Based on these findings, it has been suggested that aPL are directly able, irrespectively to the concomitant presence of SLE, to maximize the sensitization to TLR7, therefore representing additional pathogenic factors in APS setting and in aPL positive SLE patients. Using a lupus mouse model that relies on duplication of TLR7, Giannakopoulos et al. [42REF] found impaired apoptotic cell clearance when β_2GPI was knocked out. The knockout mice also demonstrated higher IFN-I signatures, higher autoantibody titers, and an exaggerated lupus phenotype as compared with the β2GPI-expressing mice [42REF]. The Authors speculated on a TLR7 inhibitory function of β2GPI in this model, which might be disrupted by aPL [42REF]

A further intriguing role of the mosaic is played by B1 cells which are expanded by EPCR-LBPA engagement and enhance the production of aPLs. B1 cells were discovered in mice over 30 years ago as a rare B lymphocyte subpopulation with unique cell surface antigens that spontaneously secretes IgM. The properties of human B1 cells have just begun to be uncovered over the past decade. B1a cells are distinct from conventional B cells (B2) by their surface marker expression and functions. Originally identified as a B-cell subset of foetal origin expressing the pan-T cell surface glycoprotein CD5, B1a cells differ from B2 by the levels of expression of several surface biomarkers (Berland Ann Rev Immunol 2: 253-300, 2002). Compared to B2, B1a are long lived and have reduced BCR diversity and affinity (Kantor AB, J Immunol 158:1175-1186, 1997). The majority of B1a cells are located in peritoneal and pleural cavities. Circulating B1 cells which are detected in adult human peripheral blood at an estimated frequency of about 1% of CD19+ve B cells derive from adult hematopoietic stem cells, which maintain the adult B1 cell pool in peripheral blood (Kageyama Y Int Journ of Hematol, 111: 628-633, 2020). They are responsible for the production of circulating IgM referred to as "natural antibodies". These low affinity antibodies are polyreactive, constitute a first line of defence against bacterial pathogens (Carroll MC Curr Opin Immunol 10:36-40, 1998) playing a pivotal role in innate immunity. This polyreactivity also results into the recognition of auto-antigens which is crucial for the clearance of apoptotic products. Due to production of these natural antibodies, which have a weak affinity for antigens, B1 cells recognize not only pathogen molecules but also self-antigens and can be produced in the absence of exogenous stimulation (Duan B, autoimmune rev, 5: 403-408, 2006, Prieto JMB, Microbiol InfectDis 54:38-44, 2017), B1a cells have been postulated to play a role in autoimmune pathogenesis, especially if the processing of nuclear debris from apoptotic cells is involved, such as systemic lupus erythematosus.

In the pathologic conditions investigated in Muller-Calleja's study (2.Science) the expansion of B1a cells responsible for the production of lipid reactive aPLs eventually triggers a vicious circle that enhances the damage mechanisms. By binding to EPCR aPLs target a toggle switch that links coagulation and innate immune signaling. As shown in Muller-Calleja's elegant experiments (2.Science), blockade of EPCR-LBPA signaling prevents the development of aPLs and remarkably attenuates renal damage in a model of TLR 7-

dependent SLE-like disease induced in mice. Therefore, an antibody blocking EPCR-LBPA not only protects mice from thrombosis and fetal loss induced by aPLs, but also interferes with the development of renal injury in an experimental model of lupus.

How can we translate these finding into clinical practise?

First, these findings might open new perspective in terms of identifying different subtypes of patients with aPL. Both SLE and primary APS have been associated with dysregulation of the type I IFN pathway, which has several putative pathogenic roles (5, 6).

Two recent studies, in which a clustering-analysis approach was employed, confirmed the presence of relatively homogeneous subgroups of aPL-positive subjects sharing similar clinical and serological characteristics corresponding to known and defined nosological entities[127,128]. At the same time, both studies also demonstrated the extreme heterogeneity of APS manifestations and the existence of an interesting subgroup of aPL-positive patients showing intermediate characteristics between SLE and PAPS with lower risk for developing thrombotic events and higher rates of systemic features such as anti-nuclear antibodies (ANA) positivity (97%) and cytopenia (mainly thrombocytopenia), supporting the idea of a *continuum* in APS clinical spectrum [128]. In line with these data, the employment of an approach based on patient profiling, rather than its mere categorization into discrete disease groups, should allow for a more real-life, precise and personalized management of these patients. Diagnostic assays, which specifically measure antibody reactivity to the pathogenic target EPCR-LBPA, have the potential to improve precision diagnostic for APS for better risk stratification and therapy in autoimmune disease.

Secondly, the study of might support some rationales for therapeutic approaches in APS beyond antithrombotic strategies. In this context, the use of HCQ, which is largely employed in both thrombotic and obstetric APS as well as a prophylactic strategy in high-risk aPL-positive subjects, has been shown to be associated with lower degrees of IFN-I activation in patients with primary APS [45REF], in line with its ability to modulate NETs formation [44REF]. Similarly, HCQ can inhibit the TLR signaling, the accumulations of nucleic fragments in the lysosomes, and the autophagic protein degradation by rising lysosomal pH.

On the other hand, the modulation of EPCR-LBPA might help in elucidating the heterogeneous results in the clinical trials investigating safety and efficacy of monoclonal anti-IFNα agents in SLE cohorts, such as sifalimumab and rontalizumab [119,120REF].

Besides, Muller-Calleja's findings on B1 cells strength the rational of empirical use of belimumab (noi, Emmi.). The observations are in line with those of Huang W etal, who

found that B cell-activating factor (BAFF) regulates the transitional B cell checkpoint, with conservation of transitional 1 (T1) cells and approximately 90% loss of T3 and naive B cells after chronic belimumab treatment. Class-switched memory B cells, B1 B cells, and plasmablasts were also substantially depleted (JCI Insight . 2018 Sep 6;3(17):e122525). Therefore, the effect of EPCR-LBPA can be potentially modulated at different levels, as it is recognized that pDCs are induced via TLR stimuli to produce IFN-α, increasing the production of BAFF, which in turns activate B cells autoreactivity [35].

However, some unmet questions remain to be elucidated.

Anti-Beta2GPI antibodies have been traditionally thought to be major biomarkers of APS (Sciascia below, Walhelm). This study hinders their role in the pathogenesis of the disease. Moreover, as already discussed, APS is not an homogeneous condition. It comprises quite different clusters of patients with SLE, with isolated thrombotic disorder or gestational troubles without serological and clinical signs of SLE, and thrombotic events in the context of an apparently systemic disorder with circulating anti-nuclear antibodies, but not SLE (Sciascia, clusters). Therefore, other mechanisms underlie the clinical expression of an aPL-mediated disease.

However, with the discovery of new innate immune cells and inflammatory mediators, innate immunity is emerging as a key player in disease pathologies. Muller-Calleja's study highlighted the importance of innate immune cells and molecules in promoting and potentiating SLE, and leads the way for new therapeutic targets as potential novel therapies in SLE. Blockade of EPCR-LBPA signaling by target monoclonal antibodies appears feasible and clinically appealing in both APS, especially the catastrophic form, and in Lupus Nephritis. Blockade could intercept the aPL-dependent- thrombotic events and hamper the renal injury, as strikingly shown in the model of TLR 7-dependent SLE-like disease induced in mice by Muller-Calleja's team.

References:

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