30 Antiphospholipid Syndrome – Experimental Models: Insight into Etiology, Pathogenesis, and Treatments

Miri Blank, Ilan Krause, and Yehuda Shoenfeld

Classical antiphospholipid syndrome (APS) – Hughes syndrome – is characterized by the presence of antiphospholipid antibodies (aPL) which bind phospholipid target molecules mainly *via* β_2-glycoprotein I (β_2-GPI), and/or lupus anticoagulant (LA) associated with recurrent fetal loss, thromboembolic phenomena, and thrombocytopenia [1–4]. Recently, accumulated evidence suggest that APS is a *systemic* autoimmune disease, associated not exclusively with coagulation failure or recurrent fetal loss but with many diverse clinical manifestations involving different organs such as the heart, the brain, the adrenal, and the skin [4–6]. Several animal models of APS have been used to address the mechanisms involved in the pathogenesis, etiology, and novel treatments. The animal models resembling APS manifestations entail: (1) MRL/lpr [7, 8] or (NZWxBxSB)F1 mice [9] which develop APS features on a genetic background; (2) passive transfer of aPL intravenously [10–13], intraperitoneally [13], or intrathecally into naive mice [14]; (3) Active immunization with aPL by idiotypic manipulation (e.g., a model based on Jerne's theory of the idiotypic network [15, 16]. Immunization of naive mice with an autoantibody (Ab1) results in generation of anti-idiotypic antibody (i.e., Ab2) followed by generation of mouse-anti-anti-idiotipic antibodies (i.e., Ab3). Ab3 may simulate Ab1 in its biological properties. The generation of Ab3 is followed by the emergence of the full-blown serological, immunohistochemical, and clinical manifestations of the respective autoimmune disease [17–20]; (4) immunization of naive mice with the autoantigen such as β_2-GPI or its synthetic derivatives [8, 21]; (5) β_2-GPI knockout mice [22]; (6) mice deficient in complement C3 [12], ICAM-I, P-selectin [23], E-selectin [24], apo-E [25], low-density lipoprotein (LDL)-receptor knockout mice [26], and SCID mice [27].

Based on studies conducted in these APS animal models, in the current chapter we will address the following topics: Reproductive failure caused by diverse autoantibodies such as direct binding aPL [e.g., cardiolipin (CL), phosphatidylserine ((PS), antibodies directed to β_2-GPI, β_2-GPI/PL, annexin V, and prothrombin]. Therapeutic approaches and mechanisms involving in modulating fetal loss in APS experimental models will be presented as well [7–55]; thrombosis caused by aPL or anti-prothrombin and its immunomodulation [55–63]; the origin of aPL and the infectious etiology of APS [64–74]; aPL involvement in the pathogenesis of brain heart and kidney [75–88]; athrosclerosisi and aPL [89–93].

Reproductive Failure and Experimental APS

An experimental model of APS was induced in naive mice by passive transfer of human polyclonal IgG fraction derived from a patient with primary APS, or a mouse aCL/β_2-GPI dependent monoclonal antibodies (mAbs) [10, 28]. The aCL/β_2-GPI were injected at different stages of pregnancy, resulting in lower fecundity rate, increased absorption index of embryos (the equivalent of human fetal loss), lower number of embryos per pregnancy, and lower mean weights of embryos and placenta. The above findings were accompanied by a prolonged activated partial thromboplastin time (aPTT) and thrombocytopenia [10, 28]. The passive transfer model was confirmed by other groups addressing different pathogenic properties of aPL such as mouse decidual necrosis [11] or placental thrombosis associated with the fetal loss [12].

Induction of mouse anti–β_2-GPI by immunization with β_2-GPI [8, 21] or by idiotypic manipulation [17–20], resulted in elevated percentage of fetal resorption, deposition of anti–CL/β_2-GPI on the affected placenta and the other experimental APS findings as described for passive transfer [10, 28].

The importance of the β_2-GPI molecule in supporting the outcome of normal pregnancy was shown previously by oral tolerance induction to β_2-GPI resulting in prevention of fetal loss [29] and recently in β_2-GPI knock out mice [22, 30]. In a serial of studies, Krilis et al [22, 30], showed the physiological requirement for functional β_2-GPI in pregnancy by evaluating reproductive outcomes in β_2-GPI–deficient mice. The study showed that although mice lacking β_2-GPI are fertile, functional β_2-GPI is essential for optimal implantation and placental morphogenesis [22, 30].

We investigated the pathogenic part in the aCL/β_2-GPI immunoglobulins responsible for the elevated fetal loss in naive mice [31]. In the past, we were able to isolate mouse monoclonal pathogenic aCL/β_2-GPI which cause reproductive failure and aCL/β_2-GPI mAbs which were not related to fetal loss from experimental APS and lupus models [18]. In order to clarify which part of the aCL/β_2-GPI Ig molecule has the pathogenic potential, we constructed and expressed several single chain Fv (scFv) of aCL/β_2-GPI, exchanging heavy and light chains between the Ig which caused fetal loss and the Ig which was not associated with reproductive failure [31]. All the expressed $scFv_s$ showed the same antigen binding properties as the original mAbs. Replacement of the fetal loss related Ig VH domain, with the non–fetal loss related VH, decreased the binding and avidity of the scFv to CL/β_2-GPI and completely abrogated the anticoagulant activity. Exchanging the pathogenic aCL/β_2-GPI VH with anti-DNA VH resulted in a shift from aCL/β_2-GPI to anti-DNA binding of the scFv. Replacement of the pathogenic aCL/β_2-GPI VL with a non-pathogenic VL, did not affect the avidity of the scFv for CL/β_2-GPI nor its anticoagulant activity. BALB/c mice were immunized with either aCL/β_2-GPI $scFv_s$, or scFv resulting from the replacement of the heavy and the light chains. The mice which were immunized with $scFv_s$ developed the same clinical manifestations, as the mice immunized with the original mAbs (elevated titers of mouse aCL/β_2-GPI , associated with LA activity, thrombocytopenia, and high percentage of fetal resorptions). Immunization with a non-pathogenic aCL/β_2-GPI–scFv did not lead to any clinical findings. Replacement of heavy/light chains between the pathogenic fetal loss related and non-fetal loss related Abs point to the importance of the heavy chain variable domains in the pathogenic potential of aCL/β_2-GPI associated with fetal loss [31].