Chapter

A Novel Autoantibody against β2-Glycoprotein I/HLA Class II Complexes in Antiphospholipid Syndrome

Kenji Tanimura, Yuki Sasagawa, Masashi Deguchi, Noriko Arase, Hisashi Arase and Hideto Yamada

Abstract

We have found that a novel autoantibody against β2-glycoprotein I (β2GPI)/human leukocyte antigen (HLA) class II complexes (anti-β2GPI/HLA-DR) is involved in the pathogenesis of antiphospholipid syndrome (APS). It was also found that many APS patients who were negative for conventional antiphospholipid antibodies (aPLs) possessed anti-β2GPI/HLA-DR. These results suggested that anti-β2GPI/HLA-DR measurements may be more sensitive for diagnosing APS than conventional aPLs tests. Recurrent pregnancy loss (RPL) is one of the clinical manifestations of APS. Therefore, a prospective, multicenter, cross-sectional study were conducted to assess whether anti-β2GPI/HLA-DR is also associated with RPL. This study of 227 couples with RPL revealed that 22.9% (52/227) of RPL women tested positive for anti-β2GPI/HLA-DR, and 24 (19.8%) of the 121 couples with unexplained RPL tested positive for anti-β2GPI/HLA-DR. Interestingly, thirty-five of the 52 (67.3%) RPL patients who were positive for anti-β2GPI/HLA-DR possessed no conventional aPLs of criteria. This novel autoantibody against β2GPI/HLA class II complexes may be a major risk factor for RPL, and it may be a promising biomarker for diagnosing APS.

Keywords: Autoantibody, β2-glycoprotein I, HLA class II, recurrent pregnancy loss

1. Introduction

It is well known that specific human leukocyte antigen (HLA) class II alleles are associated with susceptibility to many autoimmune diseases [1]. However, the mechanisms by which specific HLA class II molecules control the immune response in autoimmune diseases have been unclear. On the other hand, autoantibodies are produced in most autoimmune diseases and cause clinical manifestations of the diseases. It has also been an enigma how autoantibodies targeting self-antigens cause the autoimmune diseases. Arase *et al.* discovered a novel function of HLA class II molecules which are involved in the pathogenesis of certain autoimmune diseases [2–5].

This review will focus on the autoantibodies associating with the novel function of HLA class II molecules and the pathogenesis of antiphospholipid syndrome (APS).

2. The novel function of HLA class II molecules and autoimmune diseases

The classical function of HLA class II molecules is to present antigen peptides, derived from exogeneous proteins digested in lysosomes, to helper T-cells and by that to activate them.

Endogenous proteins, on the other hand, are formed and folded in the endoplasmic reticulum (ER). Correctly folded proteins are essential for cell survival and function. Therefore, it is believed that misfolded proteins generated in the ER are never transported to the extracellular space, because such proteins are eliminated by ER-associated degradation (ERAD).

However, Arase *et al.* discovered that misfolded proteins can be rescued from ERAD and transported to the cell surface without being processed into peptides. This process occurs in the ER via an association between the misfolded proteins and the peptide-binding groove of HLA class II molecules [2].

In addition, misfolded proteins complexed with HLA class II molecules of disease-susceptible alleles have been found to serve as targets of autoantibodies in certain autoimmune diseases, and to be involved in the disease pathogenesis. For example, immunoglobulin (Ig) G heavy chain complexed with HLA-DR and myeloperoxidase complexed with HLA-DR are major targets for autoantibodies in patients with rheumatoid arthritis and microscopic polyangiitis, respectively [3, 5].

3. The conventional concepts of antiphospholipid antibodies in APS

APS is diagnosed both by the presence of clinical manifestations, including vascular thrombosis and pregnancy morbidity, and by the presence of antiphospholipid antibodies (aPLs) which present a laboratory criteria for APS [6]. Laboratory criteria for APS include IgG and IgM anticardiolipin antibodies (aCLs), IgG and IgM anti-β2-glycoprotein I (aβ2GPI) antibodies, and lupus anticoagulant (LAC). aPLs are thought to recognize linear β2-glycoprotein I (β2GPI), which undergoes conformational changes from the circular form of β2GPI by binding to negatively charged phospholipids [7], and cause APS by interacting with vascular endothelial cells [8]. Therefore, β2GPI bound to negatively charged phospholipids or negatively charged plates is used clinically to detect autoantibodies in APS patients [9]. However, because autoantibodies against the β2GPI complexed to negatively charged phospholipids or high binding plates are detected in less than half of patients with clinical manifestations of APS [10–12], these facts suggest that additional targets of autoantibodies may exist. Furthermore, because β2GPI is a secreted protein, it cannot be universally present on the cell surface. Therefore, there might be other specific molecules which present β2GPI on the surface of vascular endothelial cells.

4. The discovery of a novel autoantibody against β2GPI/HLA-DR complex in APS

We found that 293 T cells co-transfected with β2GPI and HLA-DR expressed both β2GPI and HLA-DR on the cell surface by flow cytometry analysis

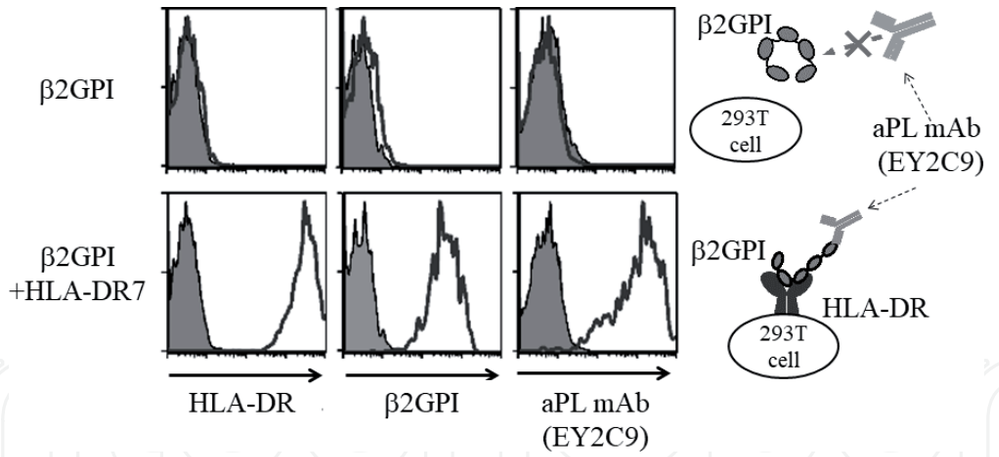

Figure 1.
Monoclonal anti-phospholipid antibody binds to β2GPI/HLA-DR complex on the cell surface. 293 T cells transfected with only β2GPI did not express β2GPI on the cell surface, and human monoclonal anti-phospholipid antibody (EY2C9) did not bind to these cells (the upper 3 histograms and 1 scheme). When β2GPI was co-transfected with HLA-DR into 293 T cells, β2GPI was expressed on the cell surface and was recognized by EY2C9 monoclonal antibody (the lower 3 histograms and 1 scheme). Abbreviations: HLA, human leukocyte antigen; β2GPI, β2-glycoprotein I; aPL mAb, anti-phospholipid monoclonal antibody.

(**Figure 1**) [4]. Conversely, 293 T cells transfected with only β2GPI did not express β2GPI on the cell surface, because β2GPI is a secreted protein (**Figure 1**) [4]. Immunoprecipitation and immunoblotting experiments revealed that full-length β2GPI proteins, but not peptide fragments of β2GPI, formed a complex with HLA-DR, and that these full-length β2GPI/HLA-DR complexes were present on the cell surface [4].

Furthermore, flow cytometry analysis revealed that not only the monoclonal antiphospholipid antibody derived from an APS patient (EY2C9), but also antibodies in the sera of APS patients can bind to the β2GPI/HLA-DR complexes, even in the absence of phospholipids [4].

5. Autoantibodies targeting β2GPI/HLA-DR complex are involved in the pathogenesis of APS

Immunofluorescence staining and *in situ* proximity-ligation assay (PLA), which detect close proximity (less than 40 nm) between two molecules [13], showed that β2GPI and HLA-DR were co-localized in endothelial cells of the placental decidua vessels from APS patients with spontaneous abortion. In contrast, no co-localization of β2GPI and HLA-DR was observed in placental tissues obtained from patients without APS [4].

In addition, we found that monoclonal antibody EY2C9 exhibited complement-mediated cytotoxicity against 293 T cells expressing β2GPI together with the APS susceptibility allele HLA-DR7, however the cytotoxicity was not detected against 293 T cells expressing HLA-DR7 alone or against those transfected with β2GPI alone [4].

HLA class II expression on endothelial cells is known to be induced after exposure to cytokines, such as IFN-γ and TNF-α [14]. Therefore, inflammatory stimuli can induce HLA class II expression on vascular endothelial cells, and HLA class II molecules transport structurally altered β2GPI, which has high affinity for the peptide-binding grooves of the alleles of HLA class II. Autoantibodies against β2GPI/HLA class II complexes may damage vascular endothelial cells expressing β2GPI/HLA class II complexes in a complement-dependent manner and cause clinical manifestations of APS, including vascular thrombosis and pregnancy

complications. In this way, β2GPI/HLA class II complexes and autoantibodies against the complexes may be involved in the pathogenesis of APS.

6. Alleles of HLA-DR complexed with β2GPI affect susceptibility to APS

HLA-DR4, HLA-DR7, and HLA-DR13 have been reported as susceptibility alleles for APS [15–18]. However, the mechanism by which these HLA class II alleles increase susceptibility to APS has remained an enigma.

To address this issue, we analyzed the ability of different HLA-DR alleles to transport β2GPI to the cell surface and found that HLA-DR7 and HLA-DR4 could transport much higher levels of β2GPI than other HLA-DR alleles recognized by the EY2C9 monoclonal antibody [4]. These results indicated that a binding affinity of β2GPI to each HLA-DR allele is important for autoantibody recognition of β2GPI/HLA-DR complexes and is associated with differences in susceptibility to APS between different HLA-DR alleles.

7. A method for quantifying serum levels of autoantibodies against β2GPI/HLA-DR complexes

We developed and modified a method to measure serum levels of autoantibodies against β2GPI/HLA-DR complexes (anti-β2GPI/HLA-DR) [4, 19].

Green fluorescent protein (GFP)-labeled β2GPI/HLA-DR complex-expressing 293 T cells and DsRed-labeled HLA-DR-expressing 293 T cells were generated by transient transfection [19]. A serum sample from a patient in whom anti-β2GPI/HLA-DR were detectable after a 10^6-fold dilution was used as a standard serum. The anti-β2GPI/HLA-DR level of a standard serum was defined as 1,000 units. The mean fluorescence intensity (MFI) of IgG binding to transfected cells in the sample sera was analyzed by flow cytometry. Specific IgG binding to the β2GPI/HLA-DR complex was calculated by subtracting the MFI of IgG binding to HLA-DR-expressing cells from β2GPI/HLA-DR complex-expressing cells. Serum levels of anti-β2GPI/HLA-DR in each sample were calculated from the standard curve generated by measuring specific IgG binding to the β2GPI/HLA-DR complex in serially diluted standard serum.

8. Autoantibody against β2GPI/HLA-DR complex is a promising novel biomarker for APS

In our previous study, we measured serum levels of anti-β2GPI/HLA-DR in stored sera from 120 patients with APS, most of whom had a history of vascular thrombosis, and found that 83% of the 120 patients had autoantibodies directed against β2GPI/HLA-DR complexes. Furthermore, about 50% of the APS patients who tested positive for anti-β2GPI/HLA-DR (< 99th percentile values measured in sera of 100 healthy subjects) were negative for both IgG aCLs and IgG aβ2GPI antibodies [4]. Another recent study also showed that 27% of 111 patients with idiopathic chronic limb ulcers who were negative for aPLs possessed anti-β2GPI/HLA-DR [20]. These results suggest that anti-β2GPI/HLA-DR are associated with APS manifestations, even in patients who do not meet the diagnostic criteria for APS because they are negative for conventional aPLs.

The latest prospective, multicenter, cross-sectional study, of 227 couples with recurrent pregnancy loss (RPL), which is one of the clinical manifestations of APS,

revealed that 22.9% (52/227) of women with RPL tested positive for anti-β2GPI/HLA-DR (< 99th percentile values measured in sera of 208 healthy, fertile control women) [19]. In this study, anti-β2GPI/HLA-DR were detected most frequently in women with RPL among other commonly recognized risk factors for RPL, i.e., uterine malformation, thyroid dysfunction, chromosomal abnormality, aPLs positive, low factor XII activity, low protein S activity, and low protein C activity (**Figure 2**). Importantly, 53.3% (121/227) of women with RPL had no commonly accepted risk factors for RPL, and 24 of these 121 (19.8%) women with unexplained RPL were positive for anti-β2GPI/HLA-DR (**Figure 2**). In addition, 45 of the 227 women with RPL (19.8%) were positive for at least one of the 5 conventional aPLs meeting the diagnostic criteria for APS in this study, i.e., IgG aCL (8.8%), IgM aCL (6.2%), IgG aβ2GPI (3.1%), IgM aβ2GPI (1.3%), and LAC (2.6%). The rate of positivity for anti-β2GPI/HLA-DR was the highest (22.9%) of the 5 aPLs that met the diagnostic criteria for APS. Notably, 35 (67.3%) of the 52 women with RPL who were positive for anti-β2GPI/HLA-DR, were negative for APS laboratory criteria (**Figure 3**).

On the other hand, the presence of multiple aPLs and LAC positivity has been reported to be strongly associated with the severity of clinical manifestations of APS [21–26]. In our study, all 3 women with RPL who had double or triple aPLs positivity were also positive for anti-β2GPI/HLA-DR, and the 2 with triple positivity had very high anti-β2GPI/HLA-DR levels (927.5 units and 330.7 units). First of both women experienced early-onset HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets) at 14 weeks of gestation, and the second experienced a thromboembolism with cerebral infarction [19]. Multiple positivity for aPLs may be associated with higher levels of anti-β2GPI/HLA-DR, and these conditions may be closely associated with the severity of the clinical manifestations of APS.

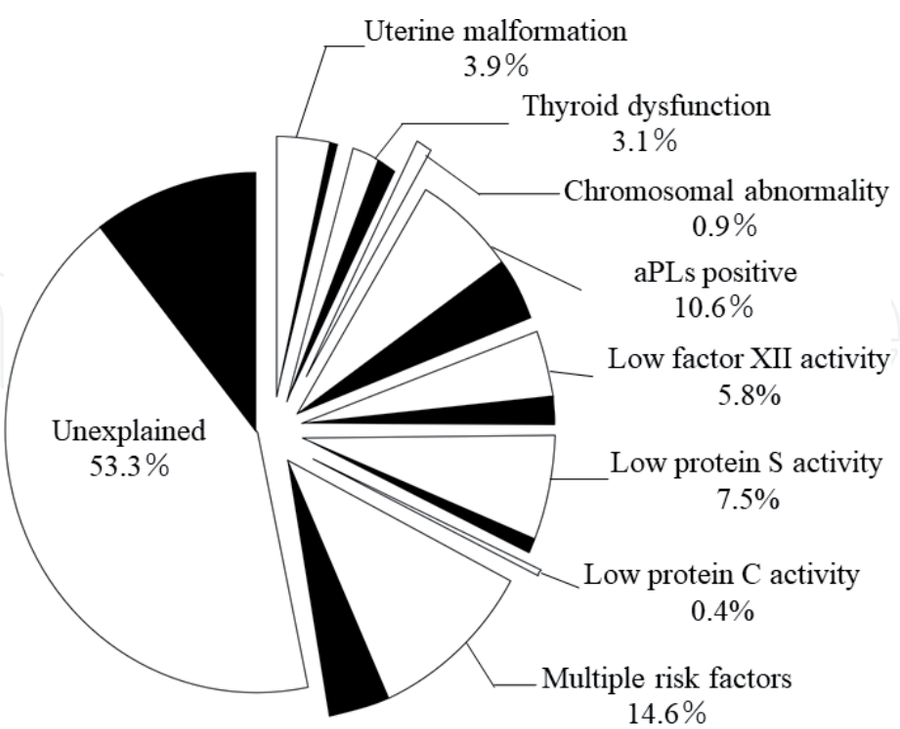

Figure 2.
Risk factors for recurrent pregnancy loss (RPL) among 227 women with RPL. All women with RPL enrolled in this study attended evaluations to identify commonly accepted risk factors for RPL. Black pie slices indicate the frequencies of women with RPL who were also positive for anti-β2GPI/HLA-DR (n = 52). Abbreviations: aPLs, antiphospholipid antibodies.

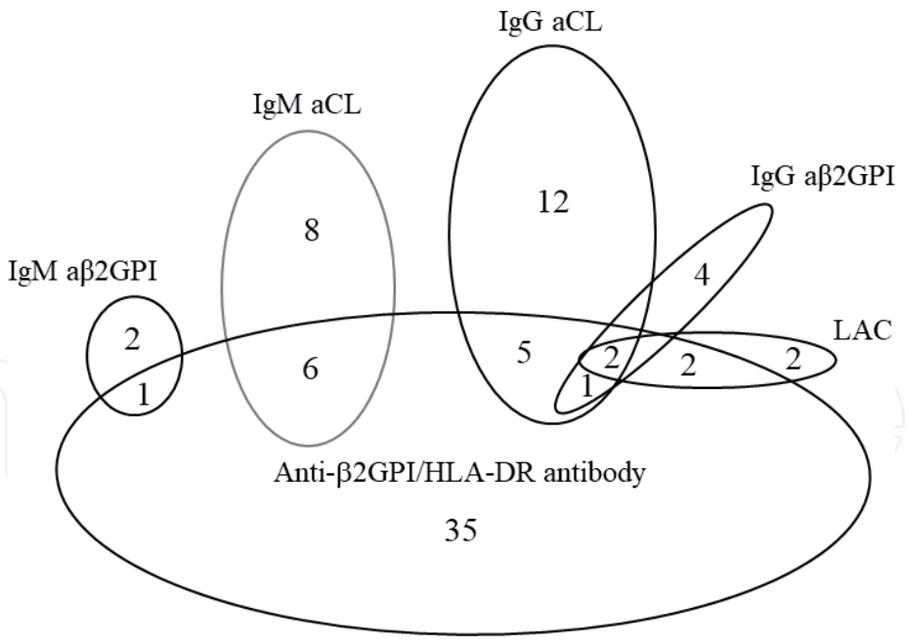

Figure 3.
Positivity for anti-β2-glycoprotein I /HLA-DR antibodies (anti-β2GPI/HLA-DR) and antiphospholipid antibodies (aPLs) in 227 women with recurrent pregnancy loss (RPL). Numbers in the Venn diagram represent the number of women who had unique or nonunique results in tests for aPLs and anti-β2GPI/HLA-DR. abbreviations: Ig, immunoglobulin; HLA, human leukocyte antigen; β2GPI, β2-glycoprotein I; aβ2GPI, anti-β2-glycoprotein I antibody; aCL, anti-cardiolipin antibody; LAC, lupus anticoagulant.

9. The future perspectives of the clinical use of autoantibodies targeting β2GPI/HLA-DR complexes

The standard treatment for pregnant women with APS is combination therapy with heparin and low-dose aspirin (LDA) [27], and the same therapy could also be effective in the treatment of women with RPL and anti-β2GPI/HLA-DR positivity. A cohort study is already underway to assess the efficacy of LDA and/or heparin therapy in such women. The history of vascular thrombosis and obstetric complications, including hypertensive disorders of pregnancy and fetal growth restriction, has not been investigated in prospective studies. Future studies assessing whether anti-β2GPI/HLA-DR are associated with thrombosis, hypertensive disorders of pregnancy, and fetal growth restriction are needed.

Further understanding of these novel autoantibodies associated with novel function of HLA class II molecules will provide new insights into the etiology of not only APS but also other autoimmune diseases and might lead to development of new treatment strategies for these diseases.

Author details

Kenji Tanimura[1,2], Yuki Sasagawa[1,2], Masashi Deguchi[1], Noriko Arase[2], Hisashi Arase[2] and Hideto Yamada[1*]

1 Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine, Kobe, Japan

2 Department of Immunochemistry, Research Institute for Microbial Disease, Osaka University, Suita, Japan

*Address all correspondence to: yhideto@med.kobe-u.ac.jp

References

[1] Jones EY, Fugger L, Strominger JL, Siebold C. 2006. MHC class II proteins and disease: a structural perspective. *Nat Rev Immunol* **6:**271-282.

[2] Jiang Y, Arase N, Kohyama M, Hirayasu K, Suenaga T, Jin H, Matsumoto M, Shida K, Lanier LL, Saito T, Arase H. 2013. Transport of misfolded endoplasmic reticulum proteins to the cell surface by MHC class II molecules. *Int Immunol* **25:**235-246.

[3] Jin H, Arase N, Hirayasu K, Kohyama M, Suenaga T, Saito F, Tanimura K, Matsuoka S, Ebina K, Shi K, Toyama-Sorimachi N, Yasuda S, Horita T, Hiwa R, Takasugi K, Ohmura K, Yoshikawa H, Saito T, Atsumi T, Sasazuki T, Katayama I, Lanier LL, Arase H. 2014. Autoantibodies to IgG/HLA class II complexes are associated with rheumatoid arthritis susceptibility. *Proc Natl Acad Sci U.S.A* **111:**3787-3792.

[4] Tanimura K, Jin H, Suenaga T, Morikami S, Arase N, Kishida K, Hirayasu K, Kohyama M, Ebina Y, Yasuda S, Horita T, Takasugi K, Ohmura K, Yamamoto K, Katayama I, Sasazuki T, Lanier LL, Atsumi T, Yamada H, Arase H. 2015. β2-Glycoprotein I/HLA class II complexes are novel autoantigens in antiphospholipid syndrome. *Blood* **125:**2835-2844.

[5] Hiwa R, Ohmura K, Arase N, Jin H, Hirayasu K, Kohyama M, Suenaga T, Saito F, Terao C, Atsumi T, Iwatani H, Mimori T, Arase H. 2017. Myeloperoxidase/HLA Class II Complexes Recognized by Autoantibodies in Microscopic Polyangiitis. *Arthritis Rheumatol* **69:**2069-2080.

[6] Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, Derksen RH, PG DEG, Koike T, Meroni PL, Reber G, Shoenfeld Y, Tincani A, Vlachoyiannopoulos PG, Krilis SA. 2006. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* **4:**295-306.

[7] Agar C, van Os GM, Morgelin M, Sprenger RR, Marquart JA, Urbanus RT, Derksen RH, Meijers JC, de Groot PG**.** 2010. β2-glycoprotein I can exist in 2 conformations: implications for our understanding of the antiphospholipid syndrome. *Blood* **116:**1336-1343.

[8] Giannakopoulos B, Krilis SA. 2013. The pathogenesis of the antiphospholipid syndrome. *N Engl J Med* **368:**1033-1044.

[9] Amengual O, Atsumi T, Khamashta MA, Koike T, Hughes GR. 1996. Specificity of ELISA for antibody to β2-glycoprotein I in patients with antiphospholipid syndrome. *Br J Rheumatol* **35:**1239-1243.

[10] Atsumi T, Ieko M, Bertolaccini ML, Ichikawa K, Tsutsumi A, Matsuura E, Koike T. 2000. Association of autoantibodies against the phosphatidylserine-prothrombin complex with manifestations of the antiphospholipid syndrome and with the presence of lupus anticoagulant. *Arthritis Rheum* **43:**1982-1993.

[11] Otomo K, Atsumi T, Amengual O, Fujieda Y, Kato M, Oku K, Horita T, Yasuda S, Koike T. 2012. Efficacy of the antiphospholipid score for the diagnosis of antiphospholipid syndrome and its predictive value for thrombotic events. *Arthritis Rheum* **64:**504-512.

[12] Gardiner C, Hills J, Machin SJ, Cohen H. 2013. Diagnosis of antiphospholipid syndrome in routine clinical practice. *Lupus* **22:**18-25.

[13] Soderberg O, Gullberg M, Jarvius M, Ridderstrale K, Leuchowius KJ, Jarvius J, Wester K, Hydbring P, Bahram F, Larsson LG, Landegren U. 2006. Direct observation of individual endogenous protein complexes in situ by proximity ligation. *Nat Methods* **3:**995-1000.

[14] Collins T, Korman AJ, Wake CT, Boss JM, Kappes DJ, Fiers W, Ault KA, Gimbrone MA, Jr., Strominger JL, Pober JS. 1984. Immune interferon activates multiple class II major histocompatibility complex genes and the associated invariant chain gene in human endothelial cells and dermal fibroblasts. *Proc Natl Acad Sci U.S.A* **81:**4917-4921.

[15] Savi M, Ferraccioli GF, Neri TM, Zanelli P, Dall'Aglio PP, Tincani A, Balestrieri G, Carella G, Cattaneo R. 1988. HLA-DR antigens and anticardiolipin antibodies in northern Italian systemic lupus erythematosus patients. *Arthritis Rheum* **31:**1568-1570.

[16] Hartung K, Coldewey R, Corvetta A, Deicher H, Kalden JR, Krapf F, Lang B, Lakomek HJ, Liedvogel B, Peter HH, et al. 1992. MHC gene products and anticardiolipin antibodies in systemic lupus erythematosus results of a multicenter study. SLE Study Group. *Autoimmunity* **13:**95-99.

[17] Domenico Sebastiani G, Minisola G, Galeazzi M. 2003. HLA class II alleles and genetic predisposition to the antiphospholipid syndrome. *Autoimmun Rev* **2:**387-394.

[18] Granados J, Vargas-Alarcon G, Drenkard C, Andrade F, Melin-Aldana H, Alcocer-Varela J, Alarcon-Segovia D. 1997. Relationship of anticardiolipin antibodies and antiphospholipid syndrome to HLA-DR7 in Mexican patients with systemic lupus erythematosus (SLE). *Lupus* **6:**57-62.

[19] Tanimura K, Saito S, Nakatsuka M, Nagamatsu T, Fujii T, Fukui A, Deguchi M, Sasagawa Y, Arase N, Arase H, Yamada H. 2020. The β2 -Glycoprotein I/HLA-DR Complex As A Major Autoantibody Target in Obstetric Antiphospholipid Syndrome. *Arthritis Rheumatol* **72:** 1882-1891.

[20] Arase N, Tanimura K, Jin H, Yamaoka T, Kishibe M, Nishioka M, Kiyohara E, Tani M, Matsuoka S, Ohmura K, Takasugi K, Yamamoto T, Murota H, Arase H, Katayama I. 2018. Novel autoantibody against the β2-glycoprotein I/human leucocyte antigen-DR complex in patients with refractory cutaneous ulcers. *Br J Dermatol* **178:**272-275.

[21] Pengo V, Ruffatti A, Legnani C, Testa S, Fierro T, Marongiu F, De Micheli V, Gresele P, Tonello M, Ghirarduzzi A, Bison E, Denas G, Banzato A, Padayattil Jose S, Iliceto S. 2011. Incidence of a first thromboembolic event in asymptomatic carriers of high-risk antiphospholipid antibody profile: a multicenter prospective study. *Blood* **118:**4714-4718.

[22] Ruffatti A, Del Ross T, Ciprian M, Bertero MT, Sciascia S, Scarpato S, Montecucco C, Rossi S, Caramaschi P, Biasi D, Doria A, Rampudda M, Monica N, Fischetti F, Picillo U, Brucato A, Salvan E, Vittorio P, Meroni P, Tincani A, Antiphospholipid Syndrome Study Group of Italian Society of R. 2011. Risk factors for a first thrombotic event in antiphospholipid antibody carriers: a prospective multicentre follow-up study. *Ann Rheum Dis* **70:**1083-1086.

[23] Yamada H, Atsumi T, Kato EH, Shimada S, Morikawa M, Minakami H. 2003. Prevalence of diverse antiphospholipid antibodies in women with recurrent spontaneous abortion. *Fertil Steril* **80:**1276-1278.

[24] Galli M, Luciani D, Bertolini G, Barbui T. 2003. Lupus anticoagulants are

stronger risk factors for thrombosis than anticardiolipin antibodies in the antiphospholipid syndrome: a systematic review of the literature. *Blood* **101:**1827-1832.

[25] Lockshin MD, Kim M, Laskin CA, Guerra M, Branch DW, Merrill J, Petri M, Porter TF, Sammaritano L, Stephenson MD, Buyon J, Salmon JE. 2012. Prediction of adverse pregnancy outcome by the presence of lupus anticoagulant, but not anticardiolipin antibody, in patients with antiphospholipid antibodies. *Arthritis Rheum* **64:**2311-2318.

[26] Gebhart J, Posch F, Koder S, Perkmann T, Quehenberger P, Zoghlami C, Ay C, Pabinger I. 2015. Increased mortality in patients with the lupus anticoagulant: the Vienna Lupus Anticoagulant and Thrombosis Study (LATS). *Blood* **125:**3477-3483.

[27] RPL EGGo, Bender Atik R, Christiansen OB, Elson J, Kolte AM, Lewis S, Middeldorp S, Nelen W, Peramo B, Quenby S, Vermeulen N, Goddijn M. 2018. ESHRE guideline: recurrent pregnancy loss. *Hum Reprod Open* **2018:**hoy004.