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A Novel Autoantibody against β 2-Glycoprotein I/HLA Class II Complexes in Antiphospholipid Syndrome

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Abstract

We have found that a novel autoantibody against β 2-glycoprotein I (β 2GPI)/human leukocyte antigen (HLA) class II complexes (anti- β 2GPI/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- β 2GPI/HLA-DR. These results suggested that anti- β 2GPI/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- β 2GPI/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- β 2GPI/HLA-DR, and 24 (19.8%) of the 121 couples with unexplained RPL tested positive for anti- β 2GPI/HLA-DR. Interestingly, thirty-five of the 52 (67.3%) RPL patients who were positive for anti- β 2GPI/HLA-DR possessed no conventional aPLs of criteria. This novel autoantibody against β 2GPI/HLA class II complexes may be a major risk factor for RPL, and it may be a promising bio-marker for diagnosing APS.

Keywords: Autoantibody, β 2-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 exogenous 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- β 2-glycoprotein I (a β 2GPI) antibodies, and lupus anticoagulant (LAC). aPLs are thought to recognize linear β 2-glycoprotein I (β 2GPI), which undergoes conformational changes from the circular form of β 2GPI by binding to negatively charged phospholipids [7], and cause APS by interacting with vascular endothelial cells [8]. Therefore, β 2GPI bound to negatively charged phospholipids or negatively charged plates is used clinically to detect autoantibodies in APS patients [9]. However, because autoantibodies against the β 2GPI 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 β 2GPI is a secreted protein, it cannot be universally present on the cell surface. Therefore, there might be other specific molecules which present β 2GPI on the surface of vascular endothelial cells.

4. The discovery of a novel autoantibody against β 2GPI/HLA-DR complex in APS

We found that 293 T cells co-transfected with β 2GPI and HLA-DR expressed both β 2GPI and HLA-DR on the cell surface by flow cytometry analysis

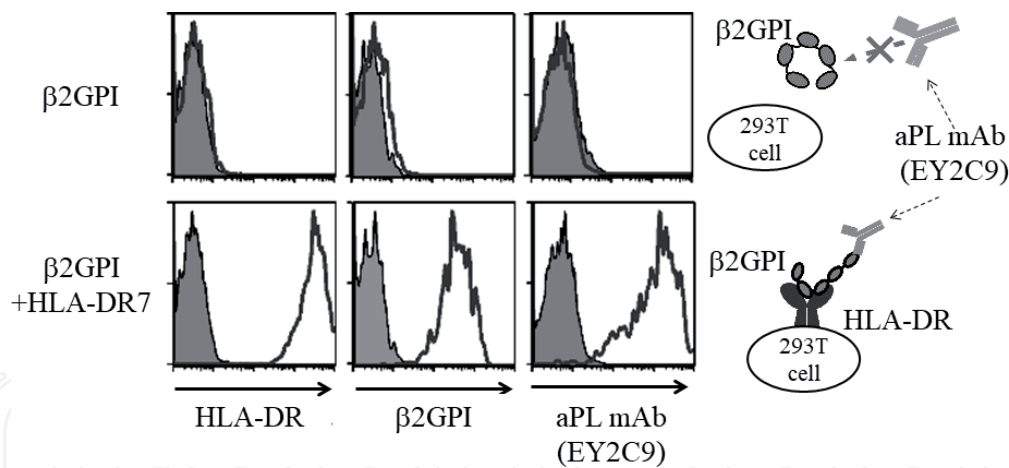


Figure 1.

Monoclonal anti-phospholipid antibody binds to β 2GPI/HLA-DR complex on the cell surface. 293 T cells transfected with only β 2GPI did not express β 2GPI 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 β 2GPI was co-transfected with HLA-DR into 293 T cells, β 2GPI 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; β 2GPI, β 2-glycoprotein I; aPL mAb, anti-phospholipid monoclonal antibody.

(**Figure 1**) [4]. Conversely, 293 T cells transfected with only β 2GPI did not express β 2GPI on the cell surface, because β 2GPI is a secreted protein (**Figure 1**) [4]. Immunoprecipitation and immunoblotting experiments revealed that full-length β 2GPI proteins, but not peptide fragments of β 2GPI, formed a complex with HLA-DR, and that these full-length β 2GPI/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 β 2GPI/HLA-DR complexes, even in the absence of phospholipids [4].

5. Autoantibodies targeting β 2GPI/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 β 2GPI and HLA-DR were co-localized in endothelial cells of the placental decidual vessels from APS patients with spontaneous abortion. In contrast, no co-localization of β 2GPI 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 β 2GPI 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 β 2GPI 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 β 2GPI, which has high affinity for the peptide-binding grooves of the alleles of HLA class II. Autoantibodies against β 2GPI/HLA class II complexes may damage vascular endothelial cells expressing β 2GPI/HLA class II complexes in a complement-dependent manner and cause clinical manifestations of APS, including vascular thrombosis and pregnancy

complications. In this way, β 2GPI/HLA class II complexes and autoantibodies against the complexes may be involved in the pathogenesis of APS.

6. Alleles of HLA-DR complexed with β 2GPI 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 β 2GPI to the cell surface and found that HLA-DR7 and HLA-DR4 could transport much higher levels of β 2GPI than other HLA-DR alleles recognized by the EY2C9 monoclonal antibody [4]. These results indicated that a binding affinity of β 2GPI to each HLA-DR allele is important for autoantibody recognition of β 2GPI/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 β 2GPI/HLA-DR complexes

We developed and modified a method to measure serum levels of autoantibodies against β 2GPI/HLA-DR complexes (anti- β 2GPI/HLA-DR) [4, 19].

Green fluorescent protein (GFP)-labeled β 2GPI/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- β 2GPI/HLA-DR were detectable after a 10^6 -fold dilution was used as a standard serum. The anti- β 2GPI/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 β 2GPI/HLA-DR complex was calculated by subtracting the MFI of IgG binding to HLA-DR-expressing cells from β 2GPI/HLA-DR complex-expressing cells. Serum levels of anti- β 2GPI/HLA-DR in each sample were calculated from the standard curve generated by measuring specific IgG binding to the β 2GPI/HLA-DR complex in serially diluted standard serum.

8. Autoantibody against β 2GPI/HLA-DR complex is a promising novel biomarker for APS

In our previous study, we measured serum levels of anti- β 2GPI/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 β 2GPI/HLA-DR complexes. Furthermore, about 50% of the APS patients who tested positive for anti- β 2GPI/HLA-DR (< 99th percentile values measured in sera of 100 healthy subjects) were negative for both IgG aCLs and IgG $\alpha\beta$ 2GPI antibodies [4]. Another recent study also showed that 27% of 111 patients with idiopathic chronic limb ulcers who were negative for aPLs possessed anti- β 2GPI/HLA-DR [20]. These results suggest that anti- β 2GPI/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- β 2GPI/HLA-DR (< 99th percentile values measured in sera of 208 healthy, fertile control women) [19]. In this study, anti- β 2GPI/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- β 2GPI/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 α 2GPI (3.1%), IgM α 2GPI (1.3%), and LAC (2.6%). The rate of positivity for anti- β 2GPI/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- β 2GPI/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- β 2GPI/HLA-DR, and the 2 with triple positivity had very high anti- β 2GPI/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- β 2GPI/HLA-DR, and these conditions may be closely associated with the severity of the clinical manifestations of APS.

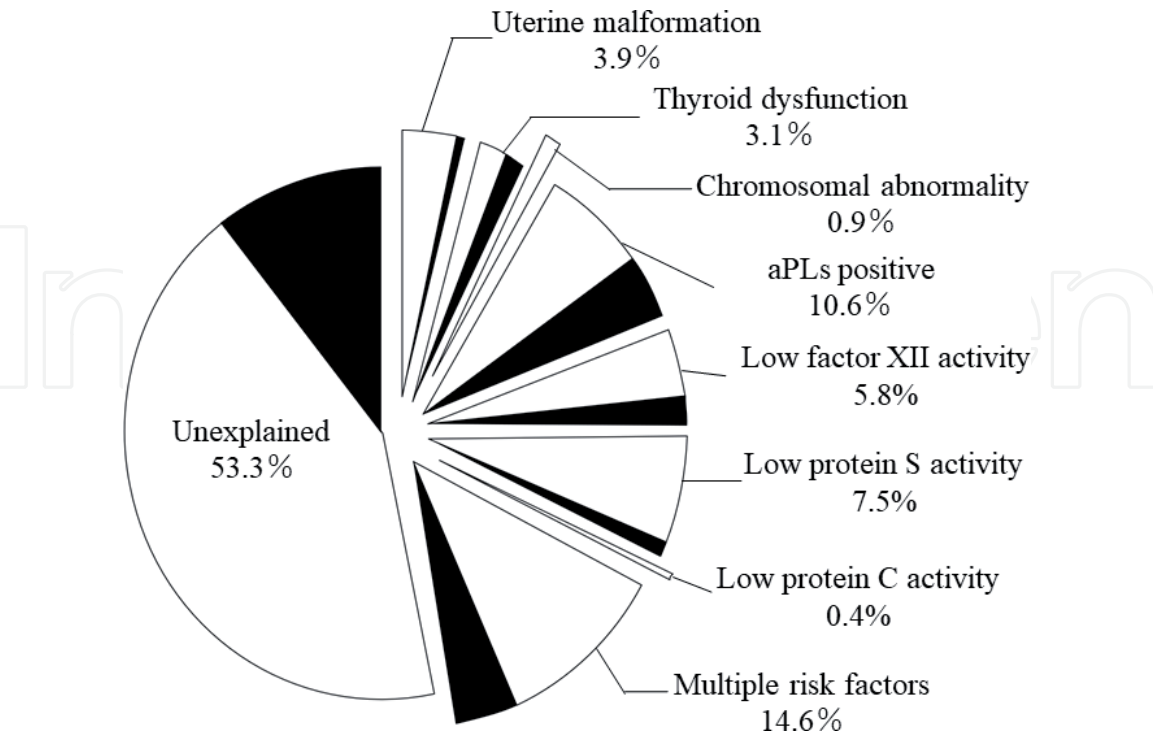


Figure 2. Risk factors for recurrent pregnancy loss (RPL) among 227 women with RPL. All women with RPL enrolled in this study attended evaluations to identify commonly accepted risk factors for RPL. Black pie slices indicate the frequencies of women with RPL who were also positive for anti- β 2GPI/HLA-DR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

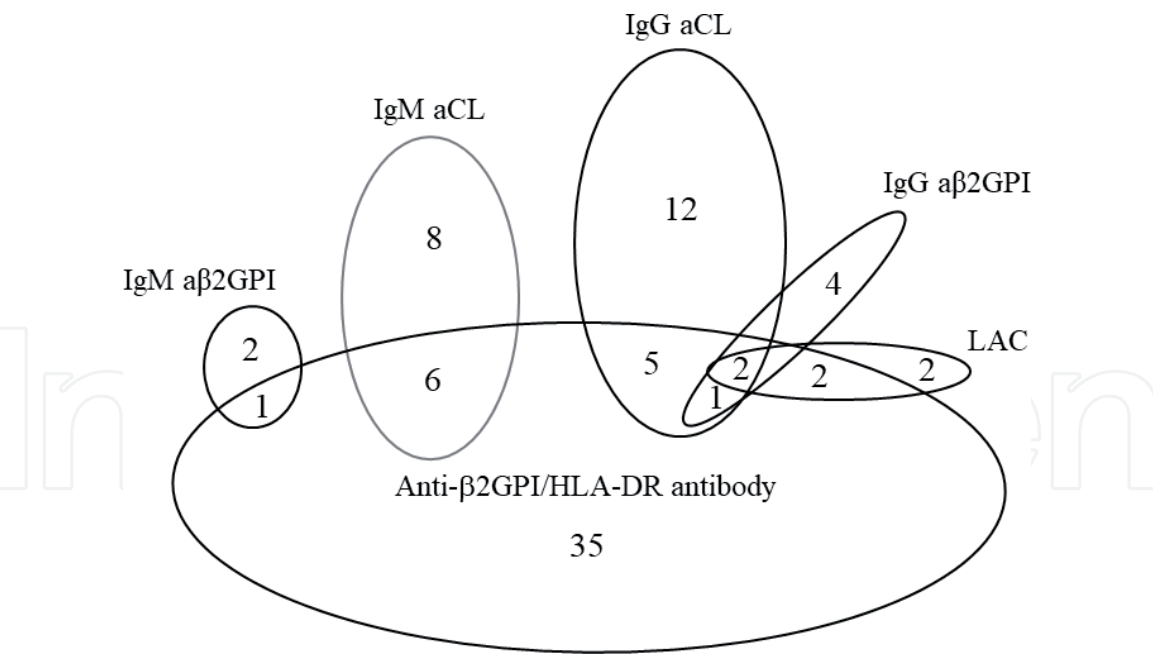


Figure 3. Positivity for anti-β2-glycoprotein I /HLA-DR antibodies (anti-β2GPI/HLA-DR) and antiphospholipid antibodies (aPLs) in 227 women with recurrent pregnancy loss (RPL). Numbers in the Venn diagram represent the number of women who had unique or nonunique results in tests for aPLs and anti-β2GPI/HLA-DR. 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.

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