1-3). Antiphospholipid Syndrome

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Introduction

Antiphospholipid antibodies (aPL) are not only the hallmark of antiphospholipid syndrome (APS), but also may play an important role in the pathogenesis of APS. Clarification of the specificity of antiphospholipid antibodies may contribute not only to the further understanding of pathological mechanisms by which the clinical features of APS are induced but also to the development of new therapeutic options against APS. Therefore, we have investigated the specificity of anticardiolipin antibodies (aCL), which are the representative pathogenic aPL, and epitopes recognized by aCL.

Antiphospholipid syndrome

In the 1980's, it was reported that aPL, such as aCL and lupus anticoagulant (LA), are related to arterial and venous thrombosis, recurrent fetal losses and thrombocytopenia in patients with systemic lupus erythematosus (1, 2). Since then, similar reports have accumulated. Furthermore, it was clarified that aPL is frequently detected in sera from patients with myocardial infarction, cerebral infarction or recurrent fetal losses who can not be classified into known autoimmune diseases (3-5). This symptomatic complex of aPL, thrombosis, recurrent fetal loss and thrombocytopenia was named the APS. Patients with APS without a known autoimmune disease are classified as primary APS (6).

Some APS patients develop multiple thrombosis, especially arterial thromboses, simultaneously, resulting in multi-organ failure. These patients often develop central nervous system disorders, such as renal dysfunction, or liver dysfunction, and are called catastrophic APS (7).

Antiphospholipid antibodies

Representative antiphospholipid antibodies are aCL, LA and antibodies responsible for biological false-positive test for syphilis (BFP-STs). These antibodies are well known as autoantibodies frequently detected in sera from patients with systemic lupus erythematosus (SLE).

Anticardiolipin antibodies have been detected by enzyme immunoassays or radioimmunoassays using solid phase cardiolipin as a target antigen. In 1990, 3 independent groups re-

ported that the binding of aCL from patients with SLE or the APS required a serum cofactor (8-10). This cofactor was proven to be β 2-glycoprotein I (β 2-GPI), a 50 kD serum apolipoprotein, by analysis of the amino acid sequence of N-terminal of the cofactor and by demonstrating the cofactor activity of recombinant human β 2-GPI (9, 11). Further studies showed that aCL from SLE patients or from APS patients did not bind to solid phase β 2-GPI on ordinary polystyrene enzyme-linked immunosorbent assay (ELISA) plates but did react with β 2-GPI immobilized on oxidized polystyrene plates (Fig. 1) (12, 13). From these results, aCL were considered to recognize cryptic epitopes which appeared on β 2-GPI, structurally altered by binding to anionic phospholipids or to oxidized polystyrene.

LA is a spectrum of autoantibodies which prolong the phospholipid-dependent coagulation tests, such as activated partial thromboplastin time test, diluted Russell's viper venom time test and kaolin clotting time test. LA was considered to be an autoantibody to phospholipids. However, recent studies suggest that LA is a group of autoantibodies against phospholipid binding proteins such as prothrombin and β 2-GPI (14, 15).

The relation of aCL and LA, so-called antiphospholipid antibodies which recognize phospholipid binding proteins, with clinical manifestations of the APS have been well demonstrated. In contrast, the clinical significance of antibodies responsible for BFP-STs in APS has not been proven. Thus, the important antiphospholipid antibodies in the diagnosis of APS are aCL and LA. aCL and LA are not only markers of APS but also

Figure 1. Antigen specificity of anticardiolipin antibodies.

may induce clinical features of APS, by altering the blood coagulability, fibrinolysis and the function of endothelium.

β2-glycoprotein I (β2-GPI)

β2-GPI, which is currently considered to be a major antigen of pathogenic aCL, is a 50 kDa plasma apolipoprotein, and consists of 326 amino acid residues and 5 N-linked oligosaccharide residues (Fig. 2). The β2-GPI is folded into 5 homologous domains called sushi domains, which correspond to the short consensus repeat (SCR) of the complement control protein superfamily. β2-GPI binds to anionic phospholipids such as cardiolipin or phosphatidylserine at the K²⁸²N²⁸³K²⁸⁴E²⁸⁵K²⁸⁶K²⁸⁷ residues of its 5th domain (16, 17). β2-GPI is reported to inhibit the ADP-induced aggregation of platelets and activation of coagulation factors, and it is considered to control thrombus formation (18–20). Recently, it was demonstrated that β2-GPI may prevent the progression of atherosclerosis (21). aCL bound to β2-GPI may inhibit these antithrombotic functions and may induce thrombosis. Analysis of peptides and epitopes recognized by pathogenic aCL may facilitate a better understanding of the pathogenesis of APS and development of new therapeutic options. For this purpose, we constructed tertiary structure model of β2-GPI and searched for the epitopes recognized by aCL.

The conformation of each domain of β2-GPI was analo-

gized based on the nuclear magnetic resonance (NMR) coordinated of the 16th domain of human factor H, replacing amino acids using conformational analysis methods (22). The 16th domain of human factor H consists of a sushi domain similar to that of β2-GPI. Alignment of all the domains was optimized by energy minimization with water molecules. The constructed model of β2-GPI is shown in Fig. 3. In this model, the domains are located around an axis forming a cylinder. The phospholipid binding site on the 5th domain of β2-GPI is found on the outer surface of this tertiary structure model.

The epitopes recognized by mouse anti-β2-GPI antibodies

In general, antibodies recognize the tertiary structure of antigen molecules. Therefore, it is difficult to analyze the epitopes based only on the amino acid sequences of the antigen. Random peptide libraries displayed on phage minor coat proteins serve as an ideal tool to identify peptides which bind to antibodies, and receptors against proteins (23). Epitopes of aCL can be presumed by searching the tertiary structure of β2-GPI for regions homologous to aCL binding peptides clarified using random peptide libraries.

To show the propriety of this strategy, the epitope of Cof-18, a monoclonal anti-human β2-GPI raised from a β2-GPI immunized mouse, was studied. According to previous studies, Cof-18 recognizes the 5th domain of β2-GPI, and inhibits

Figure 2. Amino acid sequence of human β2-GPI.

Figure 3. Tertiary structural model of β 2-GPI.

the binding of β 2-GPI to anionic phospholipids. From the study using phage display random peptide libraries, the epitope of Cof-18 was presumed at a region very close to the phospholipid binding site on the 5th domain of β 2-GPI. This result is consistent with previous studies and thus indicated that the tertiary structural model of β 2-GPI was properly constructed. This result also suggested that this strategy may be useful to study epitopes of aCL.

Epitopes recognized by β 2-GPI dependent anticardiolipin antibodies

Epitopes of EY1C8, EY2C9 and TM1G2, the monoclonal aCL established from patients with the APS, were investigated by the strategy described above. Putative epitopes which are homologous to consensus motifs of the peptides bound to these monoclonal aCL were located on the 4th domain of β 2-GPI. The 3rd domain covers these epitopes according to the tertiary structural model. These results are in agreement with our previous study which showed that pathogenic aCL do not bind to the free form of β 2-GPI (12, 13), and support the hypothesis that epitopes recognized by pathogenic aCL are exposed on β 2-GPI, when structurally altered by binding to anionic phospholipids or to oxidized polystyrene.

Conclusion

APS is a newly defined clinical entity which consists of thrombosis, fetal losses and thrombocytopenia associated with the presence of aPL. It has become apparent that the target antigens of pathogenic aPL are β 2-GPI, prothrombin, and other phospholipid binding proteins. In particular, we have identified the putative epitopes of aCL on the 4th domains of β 2-GPI. These results may contribute to the further understanding of the pathogenesis of APS and to the development of thera-

pies specifically targeting the aCL activity in APS patients.

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2-1). Antigen Peptide Analog: Induction of T cell Anergy in Primary Biliary Cirrhosis

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T cell activation is initiated upon recognition of an immunogenic peptide bound to a major histocompatibility complex (MHC) by the T cell receptor (TCR). With the aid of a costimulatory signal provided by an antigen-presenting cell (APC), T cells exert effector functions through various biologic activities of secreted lymphokines. It has recently been shown that T cell activation is not a simple on-off type event but rather qualitative changes in T cells. T cell responses can be altered by molecular mimic or amino acid substitutions of MHC molecules or antigenic peptides, *i.e.* TCR ligands, and such peptide analogs can induce the qualitative changes in T cells; T cell anergy, T cell antagonism, or changes of proliferative responses and lymphokine production (1–3). Consequently, the application of such altered peptides is expected as a treatment of autoimmune diseases (4, 5). The treatment with the altered peptide ligand is expected to selectively regulate pathogenic T cells without serious adverse effects.

Primary biliary cirrhosis (PBC) is an autoimmune liver disease of destructive intrahepatic bile ducts in which there is inflammation of the portal triads of the liver followed by fibrosis and eventually cirrhosis, then liver failure (6). In the liver of

patients with PBC, mild to intensive lymphoid infiltration, especially CD8+ as well as CD4+ T lymphocytes are seen around damaged bile ducts in the portal tract (7). Furthermore, human leucocyte antigen (HLA) class I molecules are strongly expressed and HLA-DR molecules are aberrantly expressed on biliary epithelial cells. These observations suggest that intrahepatic biliary ductular epithelial cells themselves are direct targets of an intense and highly focused immune response. However, it is uncertain how these cells actually cause damage to biliary epithelial cells and how the particular specificity of intrahepatic bile duct destruction is caused in PBC.

The characteristic immunological feature of PBC is the presence of anti-mitochondrial antibodies (AMA). Over 90% of the patients are positive for these autoantibodies. The major mitochondrial antigens recognized by AMA have been identified as the E2 components of various enzyme complexes localized to the inner mitochondrial membrane (8, 9). The immunodominant autoantigens have been defined as the E2 component of pyruvate dehydrogenase complex (PDC-E2) and the E2 component of the oxoglutarate dehydrogenase complex (OGDC-E2)(10). In the present study we generated T cell clones

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