CLINICAL RELEVANCE
OF ANTIPLATELET ANTIBODIES

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Summary

Serological methods for antiplatelet antibody determination have contributed to the understanding and better diagnosis of immune mediated thrombocytopenia. Although most tests for determination of antiplatelet antibodies were initially introduced for antibody detection in patients with idiopathic thrombocytopenic purpura, nowadays these methods are used in the diagnosis of platelet disorders when an immune pathomechanism is suspected. Serological testing is mostly used in the diagnosis of foetal and neonatal alloimmune thrombocytopenia, post-transfusion thrombocytopenic purpura and platelet transfusion refractoriness and of primary and secondary autoimmune thrombocytopenia. Development of molecular biology has enabled more accurate determination of platelet antigens and assessment of their prevalence in the population. These advancements have upgraded evaluation of the role of particular antigen systems in the etiology of auto- and alloimmune thrombocytopenic syndromes, association of human platelet antigen polymorphisms with susceptibility to cardiac and cerebrovascular diseases, and outcome of allogeneic bone marrow and solid organ transplantation.

Key words: human platelet antigens (HPA), antiplatelet auto- and alloantibodies, anti-HLA-I antibodies, immune thrombocytopenia (ITP), foetal and neonatal alloimmune thrombocytopenia (FNAITP), post-transfusion thrombocytopenic purpura (PTP), platelet refractoriness

INTRODUCTION

Platelet antigens and respective antiplatelet antibodies play a major role in the immune mediated platelet disorders. As early as the 1950s, clinical observations suggested the existence of antiplatelet antibodies. The first platelet antigen Zwa or P1A1 was discovered by Longhem in 1959, and then independently by Shulman *et al*. in 1960 in a patient with thrombocytopenia of unknown cause [1].

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Platelet antigens

Platelet antigens are an integral part of the platelet membrane glycoprotein complexes (GP IIb/IIIa, Ib/IX, Ia/IIa and Ic/IIa). The GP IIb/IIIa, GP Ic/IIa and GP Ia/IIa complexes belong to the large group of cytoadhesive proteins known as integrins; besides platelets, they are also found on leukocytes, endothelial cells and fibroblasts. Specific platelet antigens and antigens common to blood cells and other tissues, human leukocyte antigens class I (HLA-I), ABH, Le, I and P are expressed on the platelet membrane [2]. Specific human platelet antigens (HPA) have been traditionally defined as antigens found exclusively on circulating platelets and megakaryocytes. However, nowadays it is evident that most of these antigens are widely distributed in tissues and are found on the receptor molecules of the cell matrix and other cells. So, antigens located on the beta 3 integrin subunit have also been demonstrated on endothelial cells, fibroblasts and smooth muscle cells, while antigens located on the beta 2 integrin subunit are also expressed on the long-activated T lymphocytes and endothelial cells. In contrast, HPA-2 and HPA-3 antigens expressed on GP IIb and GP Ib have only been demonstrated on platelets [3]. Antigen systems have been named with numbers from 1 onward, in the order of their detection. The alleles within the system are denoted by letters, where "a" denotes high frequency allele and "b" low frequency allele. HPA polymorphism is usually a result of single nucleotide exchange, which leads to one amino acid exchange in the platelet membrane protein. This exchange causes alteration in the tertiary structure and formation of immunogenic epitopes. All known platelet specific antigens are inherited as an autosomal codominant trait. The frequency of particular genotypes/phenotypes varies greatly depending on the population and race [4].

Platelet HLA antigens are exclusively of class I, mostly A and B. HLA C antigens are very weakly expressed on platelet membrane. HLA-I expression varies considerably depending on the antigen and the individual. Until recently, it was generally considered that HLA D and DR antigens were not expressed on platelet membrane; however, the latest researches suggest that the occurrence of HLA DR antigen can be induced by cytokines (e.g., gamma interferon). ABH antigens, located on glycoprotein IIa and IIb, are in part integral membrane antigens and in part are adsorbed from plasma. Considering other antigens shared between platelets and red blood cells, Le antigens are adsorbed from plasma, whereas for example Rh antigens and other protein antigens have not been detected on platelet membrane [5].

Antiplatelet antibodies

Antiplatelet antibodies include autoantibodies, alloantibodies and isoantibodies. Autoantibodies are directed against particular glycoprotein complexes of the

own platelet membrane. Alloantibodies are formed by immunization to foreign platelet alloantigens during blood and blood product transfusion, pregnancy and transplantation. Isoantibodies are formed when the platelet membrane lacks a particular glycoprotein complex [6].

Laboratory testing for antiplatelet antibodies

The methods of antiplatelet antibody determination can be divided into three groups according to the order of their widespread application in practice. First group includes the methods based on platelet function modification as a consequence of antiplatelet antibody binding to platelet antigens (thromboagglutination test, platelet granule H^3-serotonin release assay, cytoplasmic Cr^{51} release assay and platelet factor 3 assay). These first group assays are rarely used due to their low sensitivity and specificity. Second group assays (immunofluorescence assay, radioimmunoprecipitation assay and mixed hemagglutination assay) are based on the antiglobulin assay and are used to measure IgG and/or complement complexes on platelet membranes. These assays can be direct or indirect. Direct assays measure IgG bound to patient platelets *in vivo*, whereas indirect assays determine the presence of free antibodies in patient serum. Direct assay is known as platelet associated IgG (PAIgG). The least amount of IgG (surface and intracellular) identifiable by these assays is 10-20 000 IgG molecules/platelet. Third group methods are employed to determine the presence and specificity of antiplatelet antibodies by use of monoclonal antibodies specific for the platelet glycoprotein complexes. The monoclonal antibody immobilisation of platelet antigens (MAIPA) assay is the superior third group method [1,5].

Clinical relevance of antiplatelet antibodies and platelet antigens

Direct second and third group assays (PAIgG) are most frequently employed in the serological diagnosis of immune thrombocytopenia (ITP). The validity of the assays used for determination of antiplatelet autoantibodies on platelets and/or in serum of patients with ITP remains questionable in spite of the improved method specificity and sensitivity. Problems arise on result interpretation because platelets of both healthy individuals and patients with immune mediated or non-immune mediated thrombocytopenia can be lined with IgG molecules. These assays are not mandatory in setting the diagnosis of ITP but are supplementary to other clinical-laboratory findings [7,8]. The diagnosis of foetal and neonatal alloimmune thrombocytopenia (FNAITP) is based on determination of antiplatelet antibody specificity in maternal, paternal and neonatal sera. This is accomplished by use of MAIPA and other third group assays. Maternal and paternal platelet cross-matching is useful in

detecting new antigen systems and alloantibodies to very high and low frequency antigens in the population. About 80%-90% of FNAITP cases are caused by anti-HPA-1a antibodies. Anti-HPA-5b antibodies are the second most frequent antibodies (15%), while other specific antibodies are rarely found [9, 10]. Finding compatible platelets for polytransfused patients alloimmunized to platelet antigens and refractory to platelet transfusion poses great problem to transfusion service. Refractoriness to platelet transfusion is observed in about half of the polytransfused patients. Although generally multifactorial, it frequently occurs consequentially to antibody activity. Over years, a number of methods of pretransfusion selection of platelet products have been introduced to enhance the effect of platelet transfusion [11,12]. Platelet transfusion with products prepared from HLA compatible donors, with or without previous cross-matching by the microlymphocytotoxicity test (MLCT), is most commonly used. The expected platelet count increase failed to occur in 20%-30% of patients in spite of full platelet donor and recipient HLA compatibility and negative MLCT cross-match. The concurrence of specific antiplatelet antibodies that cannot be detected by the MLCT test has been postulated in these patients. Furthermore, the variable HLA antigen expression on platelets relative to lymphocytes used in the MLCT test was the reason to turn to the use of immunofluorescence assay, enzyme-immune assay (EIA) and erythrocyte adherence assay on cross-matching prior to platelet transfusion. Platelet product selection by cross-matching before platelet transfusion yields satisfactory results of transfusion treatment in the majority of refractory alloimmunized patients. There is no generally adopted, standard cross-matching method, such as antiglobulin test in red cell serology [13]. Recently, the methods of molecular biology have been increasingly employed for platelet antigen determination. Molecular methods have enabled determination of polymorphisms and HPA allele frequency. Platelet glycoprotein polymorphism occurs due to mutation of a particular gene sequence and is the cause of hereditary thrombocytopenia [14]. The GP IIb/IIIa gene mutations cause Glanzmann thrombasthenia, while GP Ib/IX/V gene mutations cause various forms of Bernard-Soulier syndrome and thrombocytic form of pseudo von Willebrand's disease. Epidemiological studies have revealed considerable ethnic differences in the frequency of particular HPA alleles, which can prove useful on estimating the likelihood of alloimmunization to particular HPA antigens and alloimmune thrombocytopenic syndromes. Determination of the HPA system alleles in blood donors enables selection of antigen negative platelet products for the treatment of thrombocytopenic patients and patients with antiplatelet antibodies. Determination of the maternal, paternal and neonatal HPA genotype has a major role for definitive confirmation of FNAITP diagnosis. Molecular methods have been increasingly employed for determination of foetal

(prenatally) and neonatal HPA genotype from other blood cells, not exclusively from platelets, which may frequently be unavailable in the amount necessary for serological testing in case of severe thrombocytopenia. Latest researches indicate that HPA polymorphism has considerable effect on cell function and can increase susceptibility to cerebrovascular and cardiac diseases. The HPA-1b/b phenotype enhances the platelet membrane GP IIb/IIIa complex adhesive capacity and increases prothrombotic activity. In contrast, the HPA-5a/b polymorphism reduces the risk of thrombotic diseases due to functional alterations in the platelet membrane GP Ia/IIa. Although the HPA 2a/b polymorphism on GP Ib has not been demonstrated to be associated with the risk of thrombotic diseases, the antiplatelet autoantibodies in ITP patients are known to be more frequently directed against the HPA-2a than HPA-2b allotype. Incompatibility in the HPA-3 system is more commonly associated with the occurrence of acute graft-*versus*-host disease in HLA-A2 recipients. In HPA-5b negative patients, acute vascular graft rejection following allogeneic kidney transplantation has been reported. These observations suggest that HPA allotypes behave as minor histocompatible antigens [15-18].

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Sažetak

Kliničko značenje antitrombocitnih protutijela

Serološke metode za određivanje antitrombocitnih protutijela pridonijele su razumijevanju i boljoj dijagnostici imunološki uzrokovanih trombocitopenija. Iako je većina testova za određivanje antitrombocitnih protutijela u početku uvedena za dokazivanje protutijela u bolesnika s idiopatskom trombocitopenijskom purpurom, danas se ove metode primjenjuju u dijagnostici bolesti s poremećajima trombocita, kada se sumnja u imunološki patomehanizam. Serološka se ispitivanja. najčešće primjenjuju u dijagnostici fetalne i neonatalne aloimune trombocitopenije (FNAITP), poslijetransfuzijske trombocitopenijske purpure (PTP) i refraktornosti na trombocitne transfuzije, te primarne i sekundarne autoimune trobocitopenije (ITP). Razvoj molekularne biologije omogućio je točnije određivanje trombocitnih antigena i proučavanje njihove učestalosti u populaciji. Ova saznanja poboljšala su procjenu značenja pojedinih antigenih sustava u nastanku auto i aloimunih trombocitopenijskih sindroma, povezanosti HPA polimorfizma sa sklonošću srčanim i cerebrovaskularnim bolestima, te ishodu alogenične transplantacije koštane srži i solidnih organa.

Ključne riječi: humani trombocitni antigeni, antitrombocitna auto i aloprotutijela, anti-HLA-I protutijela, imuna trombocitopenija (ITP), fetalna i neonatalna aloimuna trombocitopenija (FNATP), poslijetransfuzijska trombocitopenijska purpura (PTP), refraktornost na trombocitne transfuzije

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