Review Article

Human Platelet Specific Antigens and their Importance

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Platelets are metabolically very active cells which support primary hemostasis and blood coagulation. Their glycocalyx layer on the external membrane contains a series of complex glycoprotein molecules, which are classified from glycoprotein-I to glycoprotein-X. The platelet membrane glycoproteins act as receptors for many adhesive proteins such as collagen, von-Willebrand factor, fibronectin, ADP, thrombin, *etc.* which bring about platelet aggregation. These glycoproteins express several polymorphic antigenic determinants on their surface, which are called human platelet specific antigens (HPA) (*Figs. 1a, 1b*).

HPA have conventionally been defined as antigens exclusively present on platelets and on megakaryocytes. It is now clear however, that most of these antigens have a broader tissue distribution and are expressed on receptor molecules involved in cell-matrix interactions(1). Nevertheless, the term, HPA is useful and widely used.

HPA were first described in the late 1950s and early 1960s(2). Since then, much has been learned about these biologically important platelet components. All platelet specific alloantigens are inherited by autosomal co-dominance. Their phenotype frequency varies in different racial populations. All platelet specific alloantigens so far discovered are localized on four platelet membrane glycoproteins (GP): GPIIb, GPIIIa, GPIb and GPIa.

In the Indian population studied for the frequency distribution at our center, the homozygosity of HPA-1b in the pregnant ladies was 0.65%, and the homozygosity of HPA-1a in healthy males in the reproductive age group was about 87%. Hence, it is likely that $0.87 \times 0.65 = 0.56\%$ of the ladies in the reproductive age group will be carrying a fetus positive for the incompatible HPA-1a antigen, leading to a setting in which neonatal alloimmune thrombocytopenia might develop(3).

Neonatal Alloimmune Thrombocytopenic Purpura (NAITP)

NAITP is the platelet counterpart of hemolytic disease of the newborn (HDN) and neonatal alloimmune neutropenia. In all these conditions, transplacental passage of maternal IgG alloantibodies into the fetal circulation results in increased destruction of the target blood cells. In NAITP the target blood cells are the platelets.

Pathogenesis

NAITP arises following maternal sensitization to paternal antigens present on fetal

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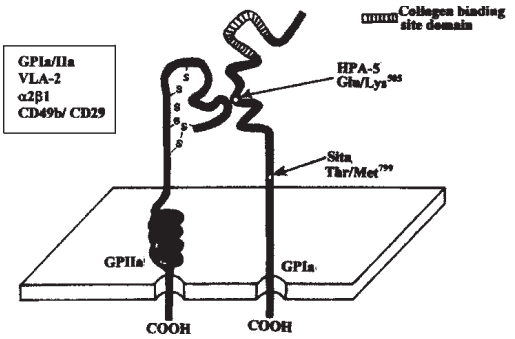

Fig. 1a. Glycoprotein Ia/IIa complex showing the polymorphic site of HPA-5 antigen (indicated by arrow). In HPA-5a/ 5b polymorphisms, amino acid Glu is substituted by Lys at position 505 in GP 1a.

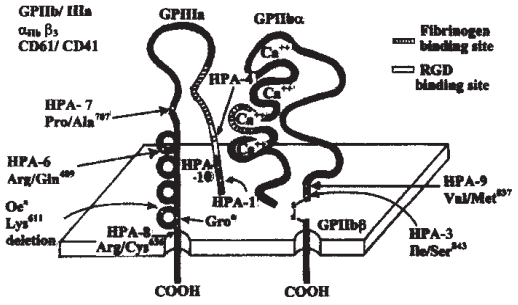

Fig. 1b. Glycoprotein IIb/IIIa complex showing the polymorphic sites of HPA-1, HPA-3, HPA-4, HPA-6, HPA-7, HPA-8, HPA-9 and HPA-10 antigens (indicated by arrow). HPA-1, 4, 6, 7,8 and 10 are expressed on GP IIIa and HPA-3 and 9 are expressed on GP IIba.

platelets(4). The maternal alloantibody produced does not react with the maternal platelets but crosses the placenta and destroys the fetal platelets by phagocytosis in the fetal reticuloendothelial system. This results in fetal thrombocytopenia. In contrast to Rh hemolytic disease, NAITP even affects the first child with thrombocytopenia(4). The severity of thrombocytopenia depends on several variables, such as (*i*) the concentration and subclass of maternal IgG alloantibodies, (*ii*) the density of the 'target' antigens on the fetal platelets, (*iii*) the activity of phagocytes in the fetal reticuloendothelial system, and (*iv*) the ability of the fetal bone marrow to compensate for the accelerated destruction of antibody- sensitized platelets(4).

In addition to ABO and HLA class I antigens, platelets express specific HPA, associated with platelet membrane glycoproteins(5). The paternal-derived fetal platelet antigen is usually HPA-1a against which the maternal alloantibody is derived. This antigen is present on the platelets of 98% of the majority of populations and is responsible for NAITP in approximately 80% of the cases(6). The second commonest platelet antigen involved in NAITP is HPA-5b (Br^a)(6,7) followed by HPA-3 (Bak). In Japanese population however, the majority of cases of NAITP are caused by antibodies to the HPA-4b (Pen^b) alloantigen(8).

Although 1-2% of most populations are HPA-1a negative it is somewhat surprising that the frequency of NAITP is lower than that would be predicted from the prevalence of the alloantigen(6,9). The most likely reason for this is that co-inheritance of certain HLA-antigens are more likely to be associated with alloantibody formation, *e.g.*, women who have the HLA-DR3 alloantigen account for the majority of affected cases of HPA-1a-induced NAITP(6,9). A similar finding is seen with the HPA-5b (Br^a) alloantigen and HLA-DRw6(6,10).

A new platelet alloantigen, Duv^{a+}, implicated in a case of NAITP has been reported recently(11). Immunochemical studies demonstrated that the epitope was localized on GPIIIa. Sequencing of the exons 2 to 15 of GPIIIa gene polymerase chain

reaction products from both parents revealed a single base substitution 517C>T (complementary DNA) present in a heterozygous state in DNA of the father leading to amino-acid substitution Thr 140 Ile (ACC>ATC) within the Arg-Gly-Asp binding domain of GPIIIa. This polymorphism does not affect significantly, the integrin function(11).

Incidence

In late 1950s and early 1960s, the incidence of NAITP reported was about one or two per 10,000 births. In 1997, the reported incidence is 1.5 per 1000 live born neonates(12). Even a frequency of NAITP of 1 in 1000 live births had been reported(13). According to our data, the risk of NAITP in India is approximately 1in 1700 pregnancies (unpublished). Using PCR technique, we have diagnosed 4 cases of NAITP from different states of India, over a period of 3 years.

Clinical Features

Generally, affected infants are often first born. The typical feature of NAITP is moderate to severe thrombocytopenia in an otherwise well neonate in the absence of any history of maternal thrombocytopenia. The most serious complication, intracranial hemorrhage (ICH) is most likely to occur during or soon after delivery; spontaneous ICH may occur *in utero* in 5-10% cases(9,14). Most reported cases of *in utero* ICH have occurred after 30 weeks gestation, but there are reports of ICH before 20 weeks(4,14). As ultrasonographic examinations are now routinely done antenatally, attention should be paid to these complications in all pregnancies and if ICH is suspected then workup for platelet antigen isoimmunization should be undertaken. In cases of suspected platelet alloimmunization, cranial ultrasound of the newborn should be undertaken as ICH might be clinically silent. Gastrointestinal bleeding, superficial hemorrhage and anemia are also relatively frequent in NAITP. The risk of bleeding is even more aggravated by impaired platelet function, which occurs as a result of antibody blocking the GPIIb-IIIa complex(15).

Mortality associated with NAITP is between 10-15%, with the majority of deaths occurring as a consequence of ICH. Neurodevelopmental sequelae in survivors of ICH are frequent and may include cerebral palsy, seizures, hydrocephalus and mental retardation(16,17). Immunization against HPA-5b usually causes milder thrombocytopenia.

Diagnosis

Neonates with NAITP usually have platelet counts of $<20 \times 10^9$/L. The diagnosis is confirmed by platelet antigen genotyping of the parents, showing the antigen to be absent on maternal platelets and present on paternal platelets or by demonstrating the presence of alloantibodies to paternal platelet-specific antigens in maternal serum(18,19). In 85% cases of NAITP, fetomaternal incompatibility for HPA-1a alloantigen is found(4). Failure to detect platelet-specific alloantibody in the maternal serum, however does not exclude the diagnosis(20,21).

Management

Antenatal management

The management of fetuses at risk of NAITP is not standardized(22). Most experts are reluctant to perform fetal blood sampling because of the dangers of this procedure. To reduce the risk of ICH in pregnancies at risk for fetal or neonatal alloimmune thrombocytopenia, various management strategies have been applied such as *in utero* fetal blood sampling, *in utero* weekly fetal

platelet transfusions, or maternal treatment with high dose intravenous immunoglobulin and/or corticosteroids along with regular ultrasonography for monitoring any fetal bleeds. In a recent review, 26 different combinations of treatment were observed(23).

As antenatal screening is currently not practiced, most cases are identified after the birth of an affected child. The question then arises which treatment option should be chosen in the next pregnancy. The recurrence rate of ICH has been reported to be as high as 90% in subsequent pregnancies. While the risk of ICH in pregnancies with thrombocytopenia, but without ICH in a previous sibling cannot be predicted, it has been estimated at 7%(24). This risk must be balanced against the risk of invasive treatment strategies.

The history of a previously affected infant with NAITP and/or the finding of circulating maternal alloantibodies during pregnancy are risk factors for NAITP in a current pregnancy(21,25). Although elective delivery before term following *in utero* transfusion of the fetus with compatible maternal platelets is of benefit(20), this approach offers no protection against very early *in utero* ICH. The most common initial site of bleeding is probably beneath the cerebral cortex, often within the temporal lobe(4). *In utero* ICH occurs most often in the third trimester. The time for fetal blood sampling recommended is 22 weeks of gestation. If the platelet count is $\leq 30 \times 10^9$/L, platelets together with intravenous immunoglobulin at 1g/ kg body weight is administered(22). At platelet count of 30-100 $\times 10^9$/L, the fetus receives only intravenous immunoglobulin once weekly. The transfusion should be slow and the fetus should be given some narcotics like remifentanil or midazolam before the transfusion. At 37 weeks' gestation, cesarean section is scheduled shortly after the last intrauterine platelet transfusion. In our experience, administration of dexamethasone phosphate (5 mg) per day to the mother from the sixth month of pregnancy till delivery reduced the blood levels of the anti-platelet antibody (unpublished data).

Postnatal management

The most effective treatment is the transfusion of antigen-negative blood-group matched platelets. The most reliable source of compatible platelets is the mother(26). If maternal platelets are transfused, it is important that they are washed and irradiated, depleted of plasma to remove pathogenic maternal alloantibodies and destroy maternal lymphocytes that are capable of stimulating transfusion associated graft-versus-host disease (GVHD)(4). If maternal platelets are not available, antigen-negative platelets from a rare blood donor panel can be selected. Management while awaiting compatible platelets, or in cases where compatible platelets are not available, is controversial and involves consideration of corticosteroid therapy, exchange transfusion, infusion of high-dose intravenous immunoglobulin or random donor platelets. High dose intravenous immunoglobulin at a dose of 0.4 g/kg daily for 5 days or 1 g/kg on 2 consecutive days is reported to be beneficial(4). High-dose intravenous immunoglobulin, is however, not as rapidly effective as compatible platelets and is less likely to be as effective in severe cases of NAITP as compared to those with passively acquired autoimmune thrombocytopenia (ITP)(27). The management is summarized in *Fig. 2*.

Post-Transfusion Purpura (PTP)

Shulman, *et al*.(28) detected antibodies for HPA-1a antigen in two patients with post-transfusion thrombocytopenia and established

PTP as a specific disorder associated in nearly all cases with an alloantibody specific for the HPA-1a (PLA1) antigen. PTP can occur in patients who are negative for one or more HPA receiving blood transfusions or platelets, which are positive for those antigens. This is a transfusion related complication and multiple transfused HPA antigen negative patients are vulnerable. PTP can result within 7-10 days after transfusion. As in NAITP, patients who

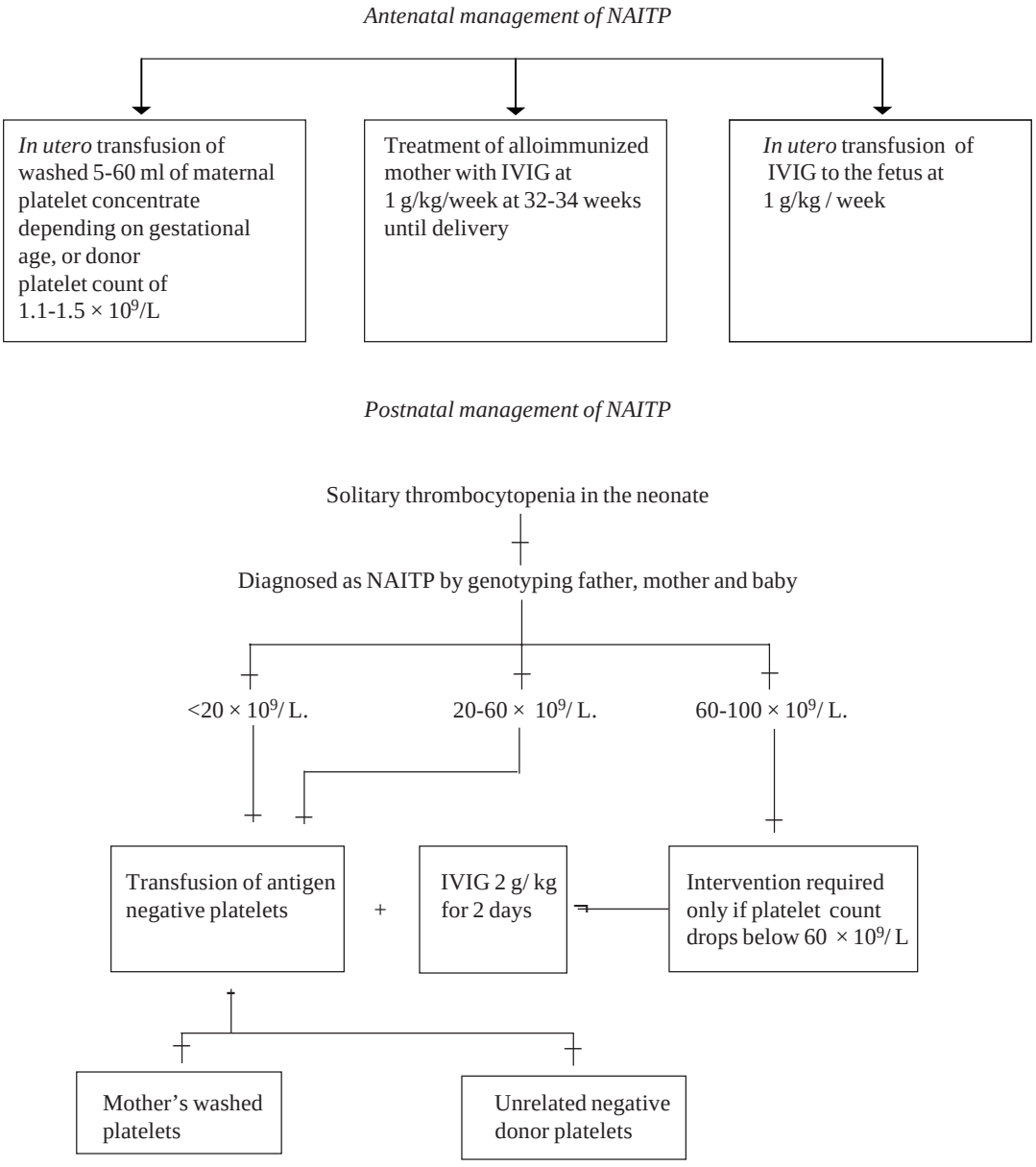

Fig. 2. Flow sheet for the antenatal and postnatal management of cases of NAITP.

Key Messages

- HPA system is one of the polymorphisms recently described in different ethnic populations.
- Several disorders have been linked to the HPA systems, including NAITP and PTP.
- Sensitization to platelet alloantigens may be influenced by another highly polymorphic antigen, the HLA system.

are HLA DR3 positive and negative for HPA-1a are more susceptible for PTP(29).

The presence of anti-HPA alloantibodies in 10 patients with beta-thalassemia major receiving periodic blood transfusions every 2-3 weeks for more than 10 yr was recently reported(30). Two out of 10 patients developed anti-HPA-2a, HPA-1b and anti-HPA-2b antibodies. These results highlight the importance of HPA alloimmunization in multitransfused patients.

In cases of PTP, antigen negative recipient platelets are also removed rapidly from the circulation. Various theories have been proposed for this phenomenon. It is proposed that during the initial phase of PTP, the recipient develops antibodies that recognize the conserved protein structures surrounding the specific polymorphic sites, which react with all the allelic forms of the antigen bearing GP. According to the second theory, the recipient antibodies form immune complexes with soluble antigens originating from the donor platelets. These complexes might interact with recipient platelets *via* an Fc-receptor dependent mechanism(31). While platelet-associated immunoglobulin (PAIg) was demonstrated during the acute phase, it disappeared with recovery and seemed to be associated with thrombocytopenia(32). According to another theory, soluble antigen from the transfused product is adsorbed onto the recipient platelets, rendering them passively positive for the HPA. The recipient antibodies then bind to his own platelets *via* this passively acquired form of autoimmunity(31).

Other clinical syndromes associated with HPA

Two other clinical conditions where the importance of platelet specific antigens is seen are Glanzmanns Thrombasthenia(33) and coronary artery disease(34).

Human Platelet Antigen-1 and Glanzmann Thrombasthenia

This is an inherited disorder of platelet function, characterized by quantitative or qualitative defects of the platelet GPIIb-IIIa complex. Patients with the condition may require repeated transfusions and therefore alloimmunization against platelet antigens is of particular interest. As GPIIIa contains HPA-1, its diminished expression in patients with Glanzmann thrombasthenia may impede serological determination of the HPA-1 allotype(32). Hence, genotyping can serve as a reliable tool for HPA-1 typing even if serological results fail. It is interesting to note that patients with Glanzmann thrombasthenia positive for HPA-1b have milder bleeding manifestations(35).

HPA and Myocardial Infarction

It has been reported that the frequency of carrying one copy of HPA-1b allele was twice higher in patients with myocardial infarction or unstable angina(36,37). However, majority

of studies have found no difference(38,39). Comparison of results of various studies is complicated by the fact that there are differences in the definition of coronary artery disease, selection of control subjects and study design. While some studies show that HPA-1b positive platelets display increased *in vitro* platelet reactivity as compared to HPA-1b negative platelets(40), others have refuted the finding(41).

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