Anti-RhD immunoglobulin in the treatment of immune thrombocytopenia

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Abstract: Immune thrombocytopenia (ITP) is an acquired bleeding autoimmune disorder characterized by a markedly decreased blood platelet count. The disorder is variable, frequently having an acute onset of limited duration in children and a more chronic course in adults. A number of therapeutic agents have demonstrated efficacy in increasing the platelet counts in both children and adults. Anti-RhD immunoglobulin (anti-D) is one such agent, and has been successfully used in the setting of both acute and chronic immune thrombocytopenia. In this report we review the use of anti-D in the management of ITP. While the FDA-approved dose of 50 mg/kg has documented efficacy in increasing platelet counts in approximately 80% of children and 70% of adults, a higher dose of 75 μg/kg has been shown to result in a more rapid increase in platelet count without a greater reduction in hemoglobin. Anti-D is generally ineffective in patients who have failed splenectomy. Anti-RhD therapy has been shown capable of delaying splenectomy in adult patients, but does not significantly increase the total number of patients in whom the procedure can be avoided. Anti-D therapy appears to inhibit macrophage phagocytosis by a combination of both FcR blockade and inflammatory cytokine inhibition of platelet phagocytosis within the spleen. Anti-RhD treatment is associated with mild to moderate infusion toxicities. Rare life-threatening toxicities such as hemoglobinuria, acute renal failure and disseminated intravascular coagulation have been reported. Recommendations have been proposed to reduce the risk of these complications. Anti-D immunoglobulin can be an effective option for rapidly increasing platelet counts in patients with symptomatic ITP.
Keywords: immune thrombocytopenia, RhD immunoglobulin

Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by a decreased number of platelets that often results in mucocutaneous bleeding.[1] ITP remains a diagnosis of exclusion. Although multiple associations have been recognized (secondary ITP), the etiology of idiopathic or primary ITP remains uncertain.[1] ITP in adults typically has a remitting-relapsing course; while ITP in children typically occurs immediately after an infectious illness and most often resolves spontaneously, without further relapses.[1,2] Secondary ITP has been associated with autoimmune disorders such as systemic lupus erythematosus, antiphospholipid antibody syndrome, or thyroid abnormalities, infections with human immunodeficiency virus, hepatitis C, or *Helicobacter pylori*, and certain hematologic malignancies.[3]

Multiple mechanisms have been described in ITP, highlighting the heterogeneous nature of this disease.[4] Anti-platelet auto-antibodies, directed against platelet membrane glycoproteins have been identified.[4] Platelets coated with IgG auto-antibodies undergo rapid clearance through Fcγ receptors on tissue macrophages.[4] Auto-antibodies may also induce megakaryocytic apoptosis resulting in defective platelet production.[4] Treatment of ITP targets different aspects of antibody-mediated platelet clearance, autoantibody production and/or immune-mediated defective platelet production.[5]

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Common first-line treatments of ITP are intended to inhibit autoantibody-mediated platelet clearance. These would include the use of corticosteroids (such as prednisone, dexamethasone), intravenous immunoglobulin (IVIg) and anti-RhD immunoglobulin (anti-D).[5] Other therapies may target autoantibody production as well as platelet clearance and can include splenectomy, danazol, azathioprine, cyclosphosphamide and anti-CD20 therapy with rituxamab.[5] Recently, romiplostim, a thrombopoietin receptor agonist, has been approved for the treatment of refractory ITP.[6] In this review we will focus on the use of anti-D immunoglobulin in the treatment of ITP.

Anti-RhD immunoglobulin

Anti-RhD immunoglobulin is composed of immunoglobulin G (IgG) prepared from the plasma of repeatedly immunized human RhD-negative donors. Anti-D IgG is manufactured from the plasma of donors and contains >90% polyclonal immunoglobulin G anti-D.[7] The concentration of IgG1, IgG2, and IgG3 are comparable to those of normal serum while the levels of IgG4 are negligible.[7,8] There are also minimal concentrations of antibodies against other red blood cell antigens.

Anti-RhD was originally prepared to prevent hemolytic disease in newborns.[9] Since 1983, it has been used to treat patients with immune thrombocytopenia.[9,10] In March of 1995, anti-D immunoglobulin was licensed by the United States Food and Drug Administration for the treatment of immune thrombocytopenic purpura in non-splenectomized children with acute ITP who were RhD-positive and children and adults with ITP secondary to human immunodeficiency infection.[8]

History of clinical use

In 1984, Salama et al published the first results on the use of intravenous anti-D in 10 Rh-positive ITP patients.[9,10] The patients had chronic ITP with the duration of their disease ranging from 1 to 21 years. Nine patients were female, of whom 4 had previous splenectomy. They received an initial intravenous dose of 200 to 1000 μg of anti-D immunoglobulin for a total dose of 300 to 3600 μg. On average, the platelets increased by 80%. The platelet counts of splenectomized patients increased by 16×10^9/L while the average increase in non-splenectomized patients was 60×10^9/L. The increased platelet count lasted from 7 to over 150 days. A transient but mild hemolysis was observed in 7 of 10 patients.

Anti-D treatment in children

In 1986, Panzer et al compared the efficacy of IVIg (0.4 g/kg × 5 days) with anti-D (11–20 μg/kg × 5 days) in 5 patients with ITP, 2 of whom were children.[11] The one child with acute ITP who received anti-D had a platelet increase of 11×10^9/L while the child with chronic ITP had a platelet increase of 22×10^9/L. Neither child had a significant decrease in their hemoglobin levels. In the same year, a collaborative study of 15 Rh-positive children with ITP were treated with low-dose anti-D (28–50 μg/kg intravenously) was reported by Becker and colleagues.[12] Ten patients had chronic ITP (duration of 6–47 months), while 5 had acute ITP. All patients had resolution of their bleeding and increased in platelet counts without a significant decrease in their hemoglobin or other adverse effects exclusive of minor transfusion related symptoms.[12] In the children with chronic ITP the duration of the platelet count increase ranged from 10 to over 360 days.

In 1991, Bussel et al treated 46 patients with ITP, of which 20 were children.[13] Anti-D was given at a treatment dose of 50 μg/kg.[13] Of the 20 children, 14 had ITP for greater than 6 months and 3 children were HIV-positive. Platelet increases were greater in children than adults (median 92×10^9/L versus 26×10^9/L). Of the 14 children with chronic disease, 2 achieved remission and 3 became stable after 5 treatments.

Andrew et al performed a multicenter cohort study of escalating doses of intravenous anti-D in children with chronic ITP (greater than 6 months' duration).[14] Twenty-five Rh-positive children received doses of anti-D that increased from 25 μg/kg upwards to 55 μg/kg. By day 7 of the protocol, 23 patients had responded to therapy, 18 of whom had platelet counts greater than 150,000/μL. The median duration of response was 5 weeks (range 1 to 24 weeks). Of the 23 responding patients, 21 were retreated and 18 responded to the second course of therapy. By the completion of the study, 16 patients continued to have excellent responses to intermittent anti-D therapy.

A randomized study of 146 children with acute ITP with platelet counts below 20,000/μL compared high dose IVIg (1 g/kg × 2 days or 0.8 g/kg × 1), intravenous anti-D (25 μg/kg × 2 days), or oral prednisone (4 mg/kg/day with tapering and discontinuation by day 21).[15] The increase in platelets was significantly faster for patients receiving IVIg compared to anti-D. The hemoglobin fell to less than 10 g/dL in 24% of the patients receiving anti-D. The authors were unable to recommend anti-D as initial therapy for children with acute ITP with platelet counts lower than 20,000/μL. Borgna-Pignatti et al published a series on 7 pediatric patients with chronic ITP who were treated with intramuscular anti-D for 5 times on an alternate-day basis.[16] Five patients responded to therapy, 2 of whom went into long-term remission. No patient experienced significant anemia despite evidence of low-grade hemolysis.

In 1997, Scaradavou et al published the largest study of anti-D use in ITP patients.[17] Two hundred seventy-two patients were treated, including 124 children and 137 adult chronic ITP patients, of which 105 patients (children and adults) were HIV-positive. An additional 11 post-splenectomy patients were also treated. The patients received 25 or 50 μg/kg/dose of intravenous anti-D, with the post-splenectomy patients receiving 100–200 μg/kg/dose. The best treatment responses were observed in the HIV-negative children. HIV-negative pediatric patients had a significantly higher mean platelet increase ($119{,}000 \times 10^9$/L) when compared to the HIV-infected children (65×10^9/L; $p = 0.005$). Also the number of responding HIV-negative children (83%) was significantly greater than that observed in the HIV-negative adults (69%; $p = 0.05$). Anti-D therapy at doses given was again shown to be ineffective in patients who failed splectectomy. The inability of the drug to work in this patient population may reflect of different pathophysiology of the disease in splenectomy failures such a antibody mediated defective megakaryopoiesis.[18]

Anti-D treatment in adults

Immune thrombocytopenia in adults is most often chronic in nature. Many patients, requiring multiple therapeutic interventions. Anti-D treatment, along with intravenous Ig, remains one of the few non-immunosuppressive therapies capable of acutely increasing platelet counts in patients with severe thrombocytopenia.

Two years following his 1984 publication, Salama reported a study of anti-D treatment in 17 adults patients with ITP (15 chronic ITP patients and 2 acute ITP).[19] The patients were given intravenous and/or intramuscular injections of anti-D. An average increase in the platelet count of 50×10^9/L was achieved in 13 of the Rh-positive patients and none of the Rh-negative patients.[19] A long-term response of greater than 5 months was achieved in 5 patients. Overt hemolysis was observed in one patient.[19] Rossi and colleagues treated 13 adult HIV-positive ITP patients with varying doses of anti-D.[20] A mean platelet count increase to greater than 100×10^9/L was observed and response was associated with a decrease in the patients' hemoglobin ranging between 1 and 1.7 g/dL.

Boughton et al reported a study of 13 Rh-positive adult patients with chronic ITP who received varying intravenous doses of anti-D.[21] Significant increases in platelet counts were seen in all patients, especially those who received 12,500 IU of anti-D. No adverse effects were observed in the patient groups. In the same year, Oksenhendler examined the effects of intravenous anti-D (12–25 μg/kg × 2 days) on 17 adult HIV-positive ITP patients with platelet counts less than 20×10^9/L.[22] Nine patients (53%) had a significant increase in their platelet counts to greater than 50×10^9/L.

In 1992, Gringeri et al performed a prospective study in 51 ITP patients, 24 of whom were HIV-positive.[23] Patients who responded to intravenous anti-D (3 μg/kg × 3 days) received intramuscular injections of anti-D (6 μg/kg/week). Twenty of the 24 HIV-positive ITP patients had a platelet response as opposed to 14 of the 27 idiopathic chronic ITP patients. In the same year, Rodeghiero et al published the results of 49 adult patients treated with high-dose IVIg or anti-D.[24] Response and remission rates did not vary significantly among the two groups.[24]

As mentioned earlier, the largest study to date was published in 1997 by Scaradavou et al.[17] In this study, 137 adult patients, including both HIV negative and positive patients, were treated with anti-D. In contrast to the results observed in the pediatric patients, HIV infection of adult ITP patients did not influence their response to anti-D. Baseline hemoglobin significantly affected platelet response to anti-D in adults with the mean platelet increase (51×10^9/L versus 21×10^9/L) and response rate (72% versus 42%) significantly higher in patients with hemoglobin levels ≥12 g/dL.

Dosing: 50 mg/kg versus 75 μg/kg

The approved dosing regimen for anti-D therapy in the United States is 50 μg/kg. However, significant questions remain as to whether higher doses may be safe and more efficacious. Newman and colleagues, reported on a randomized study comparing 75 μg/kg anti-D to the approved dose of 50 μg/kg.[25] Previous studies reported that a maximal platelet response to 50 μg/kg/d occurred 72 hours after intravenous infusion. Twenty-seven adult, HIV-negative, non-splenectomized patients with initial platelet counts less than 30×10^9/L were studied. The 75 μg/kg/day dose resulted in greater median platelet increases on days 1 and 7, when compared with the 50 μg/kg dose, without a significantly greater decrease in hemoglobin.[25]

Anti-D maintenance treatment to avoid or delay splenectomy:

Cooper et al examined whether repeated infusions of anti-D could allow adults ITP patients, who had failed initial corticosteroid therapy, avoid splenectomy.[26] Twenty-eight adult patients with platelet counts of less than 30×10^9/L were repeatedly treated with anti-D whenever their platelet count decreased to less than 30×10^9/L. A response was defined as

an incremental platelet increase greater than 20×10^9/L with an absolute value greater than 30×10^9/L within 7 days of therapy. Twenty-six (93%) patients responded to initial anti-D treatment and 19 patients (68%) responded to repeated treatments. After a median follow-up of 26 months, 19 patients avoided splenectomy; including 12 (43%) patients who were off treatment for more than 6 months without requiring a splenectomy. At study entry, a platelet count greater than 14×10^9/L was a primary predictor for patients more likely to successfully discontinue treatment with anti-D.[26]

In a second randomized study to evaluate the use of anti-D as an alternative to corticosteroid therapy in patients who wish to avoid or delay splenectomy, 70 patients were randomized to either anti-D or corticosteroid therapy. A splenectomy was to be performed if a patient failed to respond to 3 consecutive anti-D treatments given within 10 days.[27] Patients treated with repeat doses of anti-D were able to avoid splenectomy for a median duration of 112 days as opposed to 36 days in the corticosteroid treated group and required fewer days of corticosteroid therapy. However, splenectomy was performed prematurely against study protocol in 11of the 14 patients randomized to anti-D treatment and there was no statistical difference in the total number of patients in either treatment arm who subsequently had a splenectomy.[27]

Mechanisms

Multiple mechanisms whereby anti-D ameliorates thrombocytopenia have been proposed, including mononuclear phagocytic system (MPS) blockade, and cytokine modulation.[28–30]

The largest body of evidence exists for the competitive inhibition and blockade of the mononuclear phagocytic system by sensitized red blood cells within the spleen. Evidence to support this as a major mechanism of the therapy include multiple studies which show that anti-D is ineffective in RhD antigen-negative patients and relatively ineffective at standard doses in patients who have had a splenectomy. In vitro studies of cytokine expression from human monocytes and granulocytes exposed to anti-D coated red blood cells have demonstrated enhanced secretion of interleukin 1 receptor antagonist resulting in down-regulation of FcγR mediated phagocytosis.[28] Murine models of ITP have demonstrated that RBC-specific antibodies can increase platelet counts by down-regulating FcγRIIIa on splenic macrophage in a manner dissimilar to IVIg.[29]

A study evaluating the cytokine responses to infusions of IVIg and anti-D in patients with ITP demonstrated significant increases in interleukin 6 and 10, monocyte chemo-attractant protein-1, and tumor necrosis factor α within 2 hours of infusion of anti-D.[30] Higher levels of Il-10 correlated with the greatest platelet increases at 24 hours. Patients with FcγIIa-131HH genotype had higher cytokine responses and patients with FcγIIIa-158VF had higher platelet counts by day 7.[30] It would appear from these studies that anti-D therapy induces an initial inflammatory cytokine response mediated through the FcγIIa receptor leading to a secondary suppression of FcγRIIIa mediated platelet phagocytosis. This is significantly different from the anti-inflammatory effect of IVIG in which FcγIIIa and FcγIIa receptors are down-regulated by interaction of IVIG with FcγIIB receptors.[28–31]

Toxicities associated with anti-D treatment

In the original anti-D IGIV clinical trials for ITP, 2 cases of acute onset hemoglobinuria consistent with intravascular hemolysis were noted.[32] Additional cases of acute hemoglobinemia and hemoglobinuria have subsequently been reported to the FDA.[33] In April of 1998, the manufacturers of anti-D revised the package insert to include reports of hemoglobinuria that was occasionally accompanied by reversible, acute renal impairment.[8]

Gaines reviewed 11 patient reports of hemoglobinuria and noted that 7 of these patients developed significant anemia that required packed red blood cell transfusion orders.[33] Eight patients experienced new onset or an exacerbation of pre-existing renal insufficiency and 2 patients required dialysis. One patient died from pulmonary edema and respiratory distress associated with exacerbated anemia. The authors conjectured several possible mechanisms, including concomitant disorders associated with immune-mediated hemolysis such as undiagnosed Evan's syndrome; Rh phenotype with high expression of D antigen; splenic saturation and resulting diminished capacity for clearance of anti-D-sensitized RBCs; the formation of complement fixing immune complexes in vivo involving anti-D IgG1 or IgG3 aggregates with anti-idiotype antibodies in anti-D immunoglobulin that bind to the red cell initiating complement-mediated hemolysis; or improper storage and reconstitution techniques of anti-D.[33]

Gaines subsequently published a review of disseminated intravascular coagulation (DIC) reported to the FDA following anti-D administration.[34] This case series included 6 patients, 1 child and 5 adults, all of whom died. Gaines conjectured the DIC was causally related to hemoglobinemia and perhaps associated with the passive acquisition of blood group antibodies in anti-D

immunoglobulin preparation, including high-titer anti-D, low-titer anti-A, anti-B, anti-C, anti-E, anti-Duffy, and anti-Kidd antibodies.[34]

In a letter to the editor, Tarantino et al proposed a "2-hit" phenomenon responsible for the intravascular hemolysis, DIC, and the renal failure sometimes seen after anti-D administration.[35] Conditions that could have contributed to these complications with anti-D treatment include coexisting autoimmune hemolytic anemia (Evan's syndrome), antiphospholipid antibodies, pre-existing renal insufficiency, and high cytokine induced hemostatic activation.[35] The authors of the letter recommended examining for hematuria and hemoglobinuria 48 hours after anti-D infusion, screening for and avoiding use of the drug in patients with evidence of possible underlying hemolysis as suggested by an elevated reticulocyte count, and in patients at high risk for hemolysis such as those with positive direct red blood cell antibody test results.[35]

Summary

Anti-D immunoglobulin therapy has demonstrated efficacy in rapidly increasing the platelet counts in nearly 80% of children and 70% of adults with ITP. It appears to be effective only in patients with an intact spleen. However, anecdotal reports of efficacious use in splenectomized patients suggest the there may be some utility in selected patients. A more systematic study of its use in splenectomized patients may be indicated. While a higher dose of 75 μg/kg has shown in one study to result in a more rapid increase in the platelet count, it is uncertain whether this increase in the rate of response is of significant clinical benefit. Anti-D therapy appears to have significant advantages over the use of intravenous immunoglobulin in regards to expense and a longer duration of response.[17] While intermittent use of anti-D can be used to delay time to splenectomy in patients with chronic ITP, it does not appear to change the natural history of this disorder. It can allow for time for a small number of patients (15%) developing a spontaneous remission prior to splenectomy. However, it is uncertain whether use of the newer thrombopoietin receptor agonists could accomplish the same delay in splenectomy with less toxicity.[6] Finally, while severe and life-threatening toxicities are rare with anti-D therapy, recent recommended guidelines for its use can reduce the risk of these serious events.[35] Recombinant monoclonal and polyclonal anti-D antibodies have begun to be tested for autologous red cell clearance and a determination of their utility in the management of ITP awaits the results of further clinical studies.[36–37]

Disclosures

The authors have no conflicts of interest to disclose.

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